GHENT UNIVERSITY

Assessment and optimization of dietary selenium intake in Kenya: exploration of biofortification as a solution to the hidden hunger

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Assessment and optimization of dietary selenium intake in Kenya: exploration of biofortification as a solution to the hidden hunger

Nederlandstalige titel

Beoordeling en optimalisatie van selenium inname via voeding in Kenia: verkenning van biofortificatie als een oplossing voor de verborgen honger

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ABBREVIATIONS

24HR	24-hour recall
AIDS	Acquired Immune Deficiency Syndrome
Al	Aluminium
ANOVA	Analysis of Variance
BMI	Body Mass Index
Ca	Calcium
Co	Cobalt
Cr	Chromium
Cu	Copper
DDS	Dietary Diversity Scores
DOC	Dissolved Organic Carbon
DSR	Dietary Species Richness
DW	Dry Weight
EAR	Estimated Average Requirement
EELS	Electron Energy Loss Spectroscopy
Eh	Redox potential
EHS	Environmental Health and Safety
FBS	Food Balance Sheet
FCT	Food Composition Table
Fe	Iron
FW	Fresh Weight
GPx	Glutathione Peroxidase
HAZ	Height-for-Age Z-score
HIV	Human Immunodeficiency Viruses
I	Iodine
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometer
K	Potassium
LMIC	Low- and Middle-Income Country
MDD	Minimum Dietary Diversity
MDD-W	Minimum Dietary Diversity for women
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
NAR	Nutrient Adequacy Ratio

Ni	Nickel
OC	Organic Carbon
OM	Organic Matter
P	Phosphorus
PHL	Postharvest Losses
ROS	Reactive Oxygen Species
S	Sulphur
Se	Selenium
SeCys	Selenocysteine
SePP	Selenoprotein P
SSA	Sub-Saharan Africa
SeMet	Selenomethionine
WAZ	Weight-for-Age Z-score
WHO	World Health Organization
WHZ	Weight-for-Height Z-score
Zn	Zinc

SUMMARY

Selenium (Se) is an element for which trace amounts are essential for life. An adequate Se intake is crucial for antioxidant properties, redox regulation, and thyroid hormone regulation. Dietary Se intake impacts the immune system functioning, the response to viral infection, early growth and development, and the incidence of some cancers. Selenium deficiency therefore results in clinical disorders, many of them recognized today as public health problems globally. Low dietary Se intake is mainly caused by environmental conditions that inhibit soil Se mobility and availability for plants uptake resulting in low Se concentration in foodstuffs, coupled with monotonous diets based on a few staple foods. Yet, the complexity of how these factors interact and the mechanisms causing Se deficiency vary between regions and countries. Understanding these mechanisms is necessary to design suitable solutions and policies to address Se deficiency among affected communities. Selenium deficiency in Africa has previously been reported to be greatest in the East African region at 52%, with Kenya having a low dietary Se availability of 27 to 45 µg capita^{-1} day^{-1} and a risk of dietary Se inadequacy of 26 to 75 %. This thesis addressed Se deficiency in Kenya from a human nutrition, food science, environmental chemistry, and agricultural perspective. The main study region is Central Kenya Highlands, characterized by a variety of agricultural soils and a high population density relying on subsistence farming.

Chapter 1 contributes to a better understanding of the inherent background of Se deficiency in Kenya. It describes the factors that potentially contribute to the existing risk of micronutrients deficiency. It highlights that food insecurity remains a major problem with 47% of Kenyans not being able to access sufficient food to meet daily nutrients requirements especially in rural areas. This leads to the emergence of micronutrient deficiencies, as reflected in the estimated high risk of dietary Se deficiency. Notably, Se research in the developing world was constrained by analytical limitations related to costs of equipment acquisition and maintenance, and the need for specific instrumental settings and sample preparation procedures not allowing Se to be determined in a same run as other minerals. This explains unavailability of foods' Se concentration data in local food composition tables (FCTs) and consequently, its exclusion in past national nutrition surveys and interventions. Selenium is therefore not under consideration as part of health-targeted interventions and policies in Kenya. Thus, Chapter 1 elaborates on the complexity of the research problem and highlights the need to investigate the actual risk of dietary Se deficiency of the Kenyan population, and it explores potential interventional measures.

Chapter 2 presents the general methodology used in the thesis. It first describes the study region and target study population. The chapter then explains the methods that are commonly applied in subsequent chapters including dietary intake assessment, food, hair, and soil sampling methods, as well as the sample preparation and chemical analysis methods.

Chapter 3 focuses on the assessment of Se status and identification of Se-deficient locations in the Central Kenya Highlands. Based on a cross-sectional survey targeting children and women, the chapter presents the average food intake data, Se concentration of locally consumed foods, average dietary Se intake, hair Se concentration, and geochemical characteristics of various agricultural soils. The chapter further presents the Se concentration in food products grown on the local soils and Se intake-individual Se status relationships. Low mean soil Se concentration of 0.465 mg kg^{-1} and inadequate daily dietary Se intake of 14.8 µg day^{-1} for children and 32.6 µg day^{-1} for women were observed. Moreover, 87% of children and 97% of women were found to be at risk of dietary Se deficiency based on the estimated average requirements (EARs). This further related to the Se concentration in hair, whereby 92% of children and 94% of women had Se concentration levels below the reference value for human hair of 0.7 mg kg^{-1}. The chapter interprets the findings as due to over dependence on cereal grains of low Se concentration in the diet, and limited intake of good Se sources such as animal source products. An inadequate average dietary Se intake was revealed, resulting in high risk of dietary Se deficiency among the rural population. The chapter underlines the need to investigate the health burden of Se deficiency and development of intervention measures.

Chapter 4 evaluates the associations between indicators of dietary diversity and dietary Se intake, adequacy and Se status measured through hair Se concentrations in rural Kenya. No significant differences were found in food biodiversity across the study location. On average, 8 food species dominated the diets of both women and children. Less than 44% of women consumed at least 5 food groups, whereas more than 70% of children consumed maximum 4 food groups per day. In order to achieve adequate Se intake at the population level, an additional 8 and 7 food species would be required for women and children respectively, or an additional 3 to 4 food groups per day. This objective is difficult to reach considering the challenges that the rural population faces in terms of low income, dependence of rain-fed agriculture, low food production, and the impact of climate change. The chapter therefore calls for the need to address Se deficiency through a combination of population-based approaches, including diet diversification, Se biofortification, and improved soil management.

Chapter 5 presents Se agronomic biofortification experiments on maize and bean crops in the Central Kenya Highlands. It hypothesizes that agronomic biofortification is an efficient and feasible option to increase Se concentration in the staple grains and hence dietary Se intake in the population. The chapter compares the effect of soil and foliar Se fertilization at 5, 10, and 20 g Se ha^{-1} doses, on Se concentration in maize and bean grains. The study also analyses the effect of adding Se fertilizer together with phosphorus and nitrogen fertilizers in the soil application, and combining Se fertilizer with zinc and iodine fertilizers in the foliar application, on Se concentration in grains. The results show that the soil Se fertilization increased Se concentration on average by 3 µg kg^{-1} in maize and by 10 µg kg^{-1} in beans, for each gram of Se applied as sodium selenate. Foliar Se fertilization was more effective and instead increased Se concentration in grains on

average by 18 µg kg^{-1} in maize, and by 67 µg kg^{-1} in beans, for each gram of Se applied. Total soil phosphorus and its availability appeared as an important factor influencing soil Se availability. Addition of phosphorus fertilizers positively affected the impact of Se fertilization in locations with low soil P, Fe, and Al. The results further show that fertilizing beans alone was more efficient compared to fertilizing only maize. The chapter recommends that, in locations at high risk of dietary Se deficiency, foliar Se application at 10 g Se ha^{-1} on both maize and beans or 31 g Se ha^{-1} on maize alone is sufficient to achieve adequate dietary Se intake. In addition, adding Zn and I to the Se fertilizer did not affect the Se concentration in the grains. Drawing from these findings, Chapter 5 points towards application of multi-mineral agronomic biofortification, based on a site-specific biofortification strategy.

Drawing from the results in Chapter 5, Chapter 6 explores the effect of a Se biofortification intervention trial aimed at reducing the risk of dietary Se deficiency in Central Kenya Highlands. Using a cluster-randomized control trial targeting under 5-year old children and women at childbearing age, the chapter analyses the effect of foliar Se fertilization of maize crops with 20 g Se ha^{-1}, on the Se concentration in maize grains and the resultant increase in dietary Se intake. Compared to the control, the intervention had a positive and significant effect on Se concentration in maize grains of 0.448 mg kg^{-1}, corresponding to a relative difference of 94%. This resulted in a significant increase of dietary Se intake from maize grains, equal on average to 5.95 µg day^{-1} (relative difference of 103%) for the children, and to 16.4 µg day^{-1} (relative difference of 94%) for the women. Based on the overall daily foodstuff consumption at baseline and post-trial, the effect of the biofortification intervention on the average dietary Se intake of the study population was equal to 11.1 µg day^{-1} (relative difference of 96%) for children and 21.2 µg day^{-1} (relative difference of 108%) for women. The chapter concludes that biofortified maize grains improved the average dietary Se intake by 46% and 44% among children and women, while the risk of dietary Se deficiency decreased by 44% and 22%, respectively.

Finally, Chapter 7 concludes and discusses the key findings of this thesis. It also highlights some of the limitations of the study. This research examines the factors contributing to Se deficiency at the level of the soils, staple crops, and population diet, and how these three main components connect to each other. It therefore provides a very comprehensive and unique assessment of Se status situation in rural areas of the developing world. The thesis further contributes to evaluating the performance of the agronomic biofortification strategies and their potential to reduce the risk of Se deficiency in rural areas of developing countries.

SAMENVATTING

Selenium (Se) is een element dat in zeer lage concentraties essentieel is voor het leven. Hoewel het in een beperkte hoeveelheid nodig is, is een adequate Se inname cruciaal voor de werking van antioxidanten, redoxregulatie en regulatie van schildklierhormonen. Selenium inname via het dieet heeft een invloed op het functioneren van het immuunsysteem, de reactie op virale infectie, vroege groei en ontwikkeling en de incidentie van sommige kankers. Een selenium tekort kan daarom leiden tot klinische aandoeningen, die tegenwoordig algemeen worden erkend als volksgezondheidsproblemen. Inadequate Se inname via voeding wordt voornamelijk veroorzaakt door omgevingsomstandigheden die de Se mobiliteit in de bodem en diens beschikbaarheid voor de opname door planten remmen, resulterend in een lage Se concentratie in voedingsmiddelen, in combinatie met monotone eetpatronen op basis van slechts enkele basisvoedingsmiddelen. De complexiteit van hoe deze factoren samenwerken en de mechanismen die Se-deficiëntie veroorzaken, variëren echter tussen landen en regio's binnen landen, en zijn afhankelijk van het bodemtype in de landbouw en klimatologische omstandigheden. Het begrijpen van deze mechanismen is noodzakelijk om oplossingen en beleidsmaatregelen uit te werken en het Se tekort in de bevolking aan te pakken. Volgens de literatuur is Se deficiëntie in Afrika het grootst in de regio Oost-Afrika met 52%, met in Kenia een lage beschikbaarheid via voeding van 27 tot 45 μg capita^{-1} dag^{-1} en een risico op dieet-tekorten van 26 tot 75%. Dit proefschrift behandelt Se tekort in Kenia op het gebied van menselijke voeding, milieuchemie en landbouwperspectieven. De belangrijkste studie regio is het centrale hoogland van Kenia, gekenmerkt door een verscheidenheid aan landbouwbodems en een hoge bevolkingsdichtheid die afhankelijk is van zelfvoorzienende landbouw.

Hoofdstuk 1 draagt bij tot een beter begrip van de inherente achtergrond van Se-deficiëntie in Kenia. Het beschrijft de studie regio en geeft een overzicht van de factoren die mogelijk kunnen bijdragen tot het bestaande risico voor tekorten aan micronutriënten. Het benadrukt dat voedselonzekerheid nog steeds een groot probleem is, aangezien 47% van de Kenianen geen toegang heeft tot voldoende voedsel om aan de dagelijkse voedingsbehoeften te voldoen, met name in landelijke gebieden. Dit leidt tot een toename van micronutriëntentekorten, zoals weerspiegeld in het geschatte hoge risico voor Se tekorten. Onderzoek naar Se in ontwikkelingslanden kende analytische beperkingen ten gevolge van de hoge kosten van verwerving en onderhoud van analytische apparatuur, en de behoefte aan specifieke instrumentele instellingen en monstervoorbereidingsprocedures voor Se, waardoor Se niet in dezelfde runs kan worden bepaald als andere mineralen. Dit verklaart de onbeschikbaarheid van Se concentraties in voedingsmiddelen in de lokaal beschikbare tabellen voor voedselsamenstelling (FCT's) en bijgevolg ook de uitsluiting ervan in eerdere nationale voedingsenquêtes en -interventies. Selenium wordt daarom zo goed als niet overwogen bij het ontwikkelen van interventies en beleidsmaatregelen voor voeding in Kenia. Hoofdstuk 1 draagt dan ook bij aan een goed begrip

van de complexiteit van het onderzoeksprobleem. Het benadrukt de noodzaak om het feitelijke risico van een tekort aan micronutriënten in de Keniaanse bevolking te onderzoeken en onderzoekt mogelijke maatregelen.

Hoofdstuk 2 presenteert de algemene methodologie die in dit proefschrift wordt gebruikt. Eerst worden studiegebied en onderzochte bevolkingsgroepen beschreven. Het hoofdstuk legt vervolgens de methoden uit die veelvuldig toepast worden in de daaropvolgende hoofdstukken, met inbegrip van inschatting van dieet, voedsel-, haar- en grondbemonsteringsmethoden, alsook verzamelen, bewerking en analyse van de stalen.

Hoofdstuk 3 richt zich op de beoordeling van de Se-status en identificatie van Se-deficiënte locaties in de Central Kenya Highlands. Gebaseerd op een enquête gericht op kinderen en vrouwen, bevat dit hoofdstuk de gemiddelde voedingsinname gegevens, Se concentratie van lokaal geconsumeerd voedsel, werkelijke Se inname via voeding, diens Se concentratie en geochemische kenmerken van de verschillende agrarische bodems. Het hoofdstuk presenteert verder de Se concentratie in voedingsproducten geproduceerd op de lokale bodems en relaties tussen Se inname en individuele Se status. Een lage gemiddelde bodem Se concentratie van 0.465 mg kg^{-1} en een te lage Se-inname via voeding van 14.8 µg dag^{-1} voor kinderen en 32.6 µg dag^{-1} voor vrouwen worden waargenomen. Bovendien ligt de gemiddelde dagelijkse Se-inname van 87% van de kinderen en 97% van de vrouwen onder de geschatte gemiddelde behoefte (EARs). Dit relateert verder aan de Se concentratie in haar, waarbij 92% van de kinderen en 94% van de vrouwen Se concentraties hebben die liggen onder het referentieniveau voor menselijk haar (0.7 mg kg^{-1}). Het hoofdstuk interpreteert de bevindingen als een gevolg van te grote afhankelijkheid van graankorrels met lage Se concentratie in de voeding en beperkte inname van goede Se bronnen zoals dierlijke eiwitten. Een wijdverspreide ontoereikende Se inname via het dieet wordt aangetoond, wat resulteert in een hoog risico op seleniumtekorten in het dieet bij de plattelandsbevolking. Het hoofdstuk onderstreept de noodzaak om de gezondheidseffecten van Se deficiëntie en de ontwikkeling van maatregelen te onderzoeken.

Hoofdstuk 4 evalueert de associaties tussen indicatoren voor dieetdiversiteit en Se-inname via het dieet, adequaatheid en Se status gemeten via concentraties van Se in haar in ruraal Kenia. Er wordt geen significant verschil gevonden in voedingsbiodiversiteit binnen het studiegebied. Gemiddeld domineren 8 voedingsspecies het dieet van zowel vrouwen als kinderen. Minder dan 44% van de vrouwen consumeerde minstens 5 voedingsgroepen per dag, terwijl meer dan 70% van de kinderen maximum 4 voedingsgroepen per dag consumeerde. Om een adequate Se inname op populatieniveau te bereiken, zou inname van respectievelijk 8 en 7 extra voedingssoorten nodig zijn voor vrouwen en kinderen, of 3 tot 4 bijkomende voedingsgroepen. Deze objectieven zijn moeilijk te bereiken, overwegende de uitdagingen waarmee de rurale bevolking geconfronteerd wordt in termen van lage inkomens, landbouw die van regenwater afhankelijk is, lage voedselproductie en klimaatverandering. Het hoofdstuk roept dan ook op om

Se tekorten aan te pakken via een combinatie van populatie-gebaseerde benaderingen, met inbegrip van dieetdiversificatie, Se biofortificatie en verbeterd bodembeheer.

Hoofdstuk 5 presenteert agronomische Se biofortificatie-experimenten met maïs- en boongewassen in de centrale hooglanden van Kenia. Er wordt uitgegaan van de hypothese dat agronomische biofortificatie een efficiënte en haalbare optie is om de Se concentratie in de belangrijkste graangewassen en daarmee de se-inname van voedsel in de populatie te verhogen. Het hoofdstuk vergelijkt het effect van bodem- en bladbemesting aan 5, 10 en 20 g Se ha^{-1} dosissen op de Se concentratie in maïs en bonen. Het onderzoek analyseert ook het effect van het toevoegen van Se in meststof samen met fosfor- en stikstof bij grondbemesting en het combineren van Se meststof met zink- en jodiummeststoffen bij bladbemesting, op de Se-concentratie in maïs en bonen. De resultaten tonen aan dat voor elke gram Se die wordt toegepast als natriumselenaat, de Se-bemesting leidt tot een toename van de Se-concentratie met gemiddeld 3 µg kg^{-1} in maïs en 10 µg kg^{-1} in bonen. Bladbemesting is echter effectiever en verhoogt de Se-concentratie in granen gemiddeld met 18 µg kg^{-1} in maïs en met 67 µg kg^{-1} in bonen, voor elke gram Se die wordt toegepast. Totale bodemfosfor en diens beschikbaarheid blijken een belangrijke factor te zijn die van invloed is op de beschikbaarheid van bodem Se. Toevoeging van fosforhoudende meststoffen heeft een positieve invloed op de impact van Se-bemesting op locaties met weinig P, Fe en Al in de bodem. De resultaten laten verder zien dat het alleen bemesten van bonen efficiënter is dan het alleen bemesten van maïs. Het hoofdstuk beveelt aan dat, op locaties met een hoog risico op een tekort aan Se-tekorten, bladbemesting van 10 g Se ha^{-1} op zowel maïs en bonen of 31 g Se ha^{-1} op enkel maïs voldoende is om een adequate Se-inname via de voeding te bereiken. Bovendien heeft een toevoeging van Zn en I aan de Se-meststof geen invloed op de Se-concentratie in granen. Op basis van deze bevindingen behandelt hoofdstuk 5 een biofortificatiestrategie die aangepast is aan de verschillende locaties.

Op basis van de resultaten bekomen in hoofdstuk 5 onderzoekt hoofdstuk 6 het effect van een Se biofortificatie interventie gericht op de verlaging van het risico op tekorten aan seleniuminname via het dieet in de centrale hooglanden van Kenia. Gebruik makend van een cluster-gerandomiseerde interventie gericht op kinderen en vrouwen, analyseert het hoofdstuk het effect van bladbemesting van maïsgewassen aan 20 g Se ha^{-1}, op de toename van de Se-concentratie in maïskorrels en de resulterende toename van de Se-inname via het dieet. Vergeleken met de controle resulteert de interventie in een positief en significant effect op de Se-concentratie in maïskorrels van 0,448 mg kg^{-1}, hetgeen overeenkomt met een relatief verschil van 94%. Dit resulteert in een significante toename van de dieetinname van Se via mais: gemiddeld 5,95 µg dag^{-1} (relatief verschil van 103%) voor de kinderen en tot 16,85 µg dag^{-1} (relatief verschil van 94%) voor de vrouwen. Op basis van de totale dagelijkse voedselconsumptie bij baseline en post-trial, is het effect van de biofortificatie-interventie op de gemiddelde dieetinname van Se in de onderzoekspopulatie gelijk aan 10,95 µg dag^{-1} (relatief verschil van 96%) voor kinderen en 21,17 µg dag^{-1} (relatief verschil van 108%) voor vrouwen. Het hoofdstuk concludeert dat biofortificatie van maïskorrels de gemiddelde dieetinname

van Se verbetert met respectievelijk 46% en 44% bij kinderen en vrouwen. Op basis van de EARs daalde het risico op seleniumtekorten met respectievelijk 44% en 22%. Omdat er geen andere significante verschillen waren in waarneembare relevante factoren bij baseline en post-trial voor behandelingsgroepen, wordt de waargenomen afname in het risico van een tekort aan dieet-se in de interventiegroep beschouwd als voornamelijk toe te schrijven aan de Se biofortificatie-interventie.

Tot slot worden in hoofdstuk 7 conclusies getrokken en de belangrijkste bevindingen van dit proefschrift besproken. Het benadrukt ook enkele van de beperkingen van het onderzoek. Door onderzoek te doen naar de factoren die bijdragen aan Se deficiëntie op het niveau van de bodem, de gewassen en het voedingspatroon van de bevolking en hoe deze drie hoofdcomponenten met elkaar verbonden zijn, biedt dit onderzoek een zeer uitgebreide en unieke beoordeling van de Se-status in rurale gebieden van ontwikkelingslanden. Het proefschrift draagt verder ook bij tot de evaluatie van strategieën voor biofortificatie en hun potentieel om het risico op Se-deficiëntie in rurale gebieden van ontwikkelingslanden te verminderen.

CHAPTER 1: GENERAL INTRODUCTION

1.1. Background

1.1.1. Selenium in soil, plants and human health

- Selenium in the soil

Selenium (Se) is a metalloid that coexists in nature in six isotopes with mass numbers 74, 76, 77, 78, 80, and 82 (Mehdi et al. 2013). It occurs in inorganic forms as selenate (SeO_4^{2-}), selenite (SeO_3^{2-}), selenide (Se^{2-}), elemental Se, and in organic forms as selenocysteine (SeCys) and selenomethionine (SeMet), among others. In the soil, the various Se forms differ in mobility, availability, and toxicity (Gupta and Gupta 2017). In the terrestrial system, rocks are the primary source of Se whereby Se is dispersed through the food chain via complex biogeochemical cycling processes, rock-water interactions, and biological activity. Organic weathering involves plants breaking up rocks with their growing roots, while mechanical weathering consists of frost shattering when water gets into cracks and joints in bedrock. Chemical weathering results from plant acids that dissolve the rocks and mineral. Once the rock is weakened and broken up, it is eroded to form the soil in which plants grow and take up Se from the parent rocks. The distribution of Se in the geological environment therefore varies depending on the different parent rocks. As a consequence, soil Se concentration greatly varies, both locally and globally (Fordyce 2010). Selenium partitioning in the soil is further affected by pH and redox potential, content of sesquioxides, clay, organic matter (OM), and microbiological activity (Cuvardic 2003). Furthermore, Se also occurs in the atmosphere (mainly in the form of dimethylselenide) due to volatilization from volcanoes, soil, sediments, oceans, microorganisms, plants, animals, and industrial activity, and it can be transported for several thousands of kilometres before being deposited back on the earth's surface in both wet and dry forms. Accordingly, Se contents in soils are also affected by (historic) atmospheric deposition through precipitation (Haygath 1994, Haygath et al. 1995, Fordyce 2013, Blazina et al. 2014). Selenium may be redistributed depending on the mobility of Se in the soil environment, affecting the final content of Se observed in the soil. Factors affecting Se mobility in soil are discussed in the next paragraphs.

The form of Se, i.e. its speciation, and its availability and mobility in the soil are determined by a multitude of factors. In nature, Se exists in the 2^-, 0, 4^+, and 6^+ oxidation states, mainly determined by the soil pH and redox potential. Due to this complex chemistry, Se is found in all natural materials (Fordyce 2013). Both the pH and Eh play an important role in Se availability. This refers to changes in Se oxidation state and the resulting differences in chemical properties of its species as designated by the compartments depicted in the Pourbaix diagram shown in Figure 1 (Takeno 2005, Ralston et al. 2010). Under most natural redox conditions, selenite (Se^{4+}) and selenate (Se^{6+}) are the predominant oxidation states. These species occur in the oxic zone of 0.4-0.8V. At Eh within this range, changes of pH may induce transformations between selenite and selenate. At a higher pH, the Se oxidation state shifts to Se^{6+}, i.e. selenate. With all electrons in the last energy level taken, selenate is less adsorbed by soil minerals and is therefore available in soil solution. At lower

pH, the oxidation state shifts to Se^{4+} i.e. selenite. The selenite being formed is adsorbed by ligand exchange onto soil clay surfaces with greater affinity than selenate, which limits the Se concentration in the soil solution. The binding strength increases as the pH decreases (Parkman and Hultberg 2002). At lower redox potentials, i.e. in more reduced environments, these pH-dependent transformations between selenite and selenate no longer occur. Instead, transformations between selenide and elemental Se can be observed.

Along the soil profile, Se distribution resembles the distribution of Fe, Al, and clay. Because of the affinity of Se for clay minerals, soils having a higher clay content have a higher Se concentration. In an acidic environment, Se binds with the Fe, Al, and Mn oxides and hydroxides when these become positively charged. In alkaline conditions, their charge is negative. The low solubility coupled to stronger adsorption makes selenite less bioavailable than selenate. Selenate, which is the most common oxidation state in neutral and alkaline soils is soluble, mobile, and readily available for plant uptake (Neal 1995).

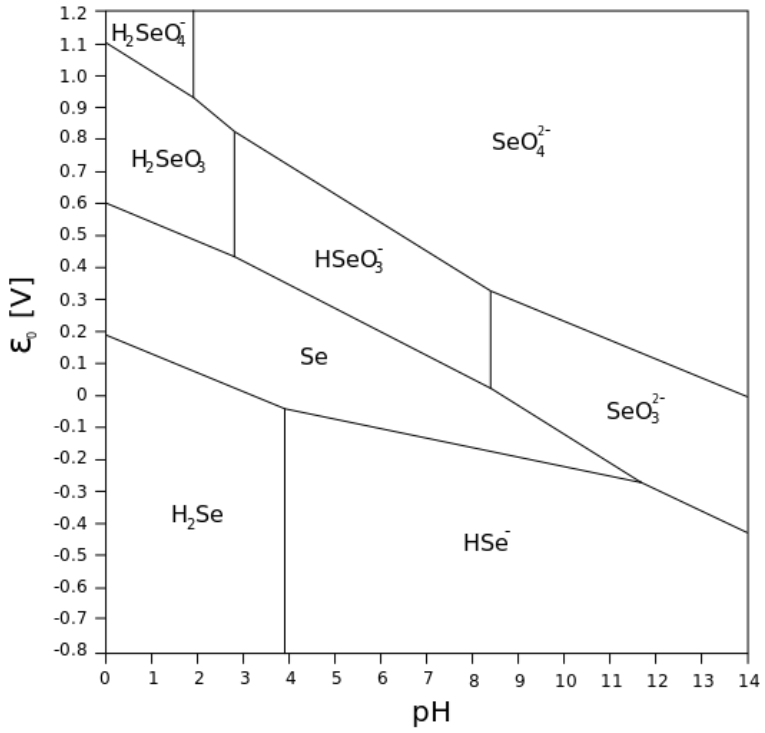

Figure 1: Pourbaix diagram for selenium (Takeno 2005, Ralston et al. 2010)

Next, the availability, mobility and uptake of Se by plants is greatly affected by the presence of competitive anions such as PO_4^{3-} and SO_4^{2-}. These anions influences Se by competing for fixation sites in the soil and plants (Goh and Lim 2004). High SO_4^{2-} in soil solution result in decreased Se uptake by plants, due to competition between SO_4^{2-} and Se for transporters in plant roots. The SO_4^{2-} ions have a greater effect on selenate than selenite because selenate uptake occurs via a sulphate transporter in the root plasma membrane. Addition of PO_4^{3-} to soils has been shown to

increase Se mobility, availability and uptake by plants as the PO_4^{3-} ion is readily adsorbed in soils and displaces selenite from fixation sites making it more bioavailable. This is because PO_4^{3-} is bound more strongly to trivalent Fe and Al than is selenite. Conversely, increasing the levels of PO_4^{3-} in soil can also decrease the Se content in the vegetation by inducing increased plant growth, resulting in dilution of Se in the crops (Jacobs 1989, Maryland 1994, Neal 1995).

Furthermore, speciation, mobility and availability of Se in soil are also affected by organic matter and related microbial activity. Selenium content typically decreases with depth (particularly in acidic soils), because it binds with proteins and other organic compounds in the upper soil layer. Selenium fixation in the organic matter fraction is possible through adsorption processes or through microbiological incorporation into amino acids and other Se-containing organic compounds. Microorganisms transform the selenite into organic compounds or into selenate. These microorganisms may also induce reducing conditions, transforming selenate into less mobile selenite in soils rich in carbon. Furthermore, the products of microbiological activity can also be volatile, which causes Se depletion from the soil (Christophersen et al. 2012).

Finally, rainfall also influences speciation, mobility and availability of Se in soil. In humid regions and acidic soils, the prevailing form is selenite. High annual precipitation results in reducing conditions in water-saturated soil which induces the (microbial) transformation of selenate into selenite. However under well-aerated conditions in the alkaline soils of semiarid regions, Se is present in the form of selenate (Cuvardic 2003). Selenium can also be deposited on the soil through rainfall (Blazina et al. 2014). Wet deposition in the form of rain or snow accounts for 76-93% of total Se deposition with more than 70% of the Se being available in a soluble form (Haygath 1994, Haygath et al. 1995, Fordyce 2013).

In general, the underlying geology and rainfall are primary determinants of Se inputs in the soils, while its speciation, mobility and availability in the soils are influenced by biophysicochemical parameters such as soil pH and redox conditions, soil texture and mineralogy, microbiological activity, and the presence of competing anions. These factors will affect not only the final contents of Se observed in the soils, but also its availability to plants. The behavior of trace elements in the environment is therefore determined by their specific physiological forms rather than their total concentration and consequently, a wide range of chemical speciation and fractionation methods have been developed and applied (Du Laing 2010).

- Selenium in the plants

Selenium enters the food chain through plants and the Se amount in food reflects Se levels in the environment or in the soil where crops are grown (Li et al. 2014). Selenium distribution in plants depends on the concentration of Se supplied to the roots and on the nature and concentration of other substances in the root zone. For instance, selenate competes with sulphate for uptake by plants, as both anions are taken up via a sulphate transporter in the root plasma membrane. Sulphate salinity therefore inhibits selenate uptake by plants (Terry et al. 2000). Unlike selenate,

there is no evidence that the uptake of selenite is mediated by membrane transporters (Arvy 1993, Tarun et al. 2000). The Se species taken up by plants determines also its translocation from root to shoot. Selenate is for instance transported more easily compared to selenite or organic Se (Arvy 1993, Zayed et al. 1998). Plants therefore transport and accumulate selenate in the leaves rather than selenite or SeMet. Selenite is poorly translocated to shoots because it is rapidly converted to SeMet, which is retained in the roots (Zayed et al. 1998). Plants convert Se mainly into SeMet and incorporate it into protein in place of methionine (Met). SeMet can account for 50% of the total Se content of the plant, whereas SeCys, methyl-selenocysteine and γ-glutamyl-Se-methylselenocysteine are not significantly incorporated into plant protein and are relatively low irrespective of soil Se content (Vendeland et al. 1994). Selenium is incorporated into the plant structure depending on the plant species, phase of development, and physiological condition. For Se accumulators, Se is accumulated in young leaves during the early vegetative stage of growth, while high Se content is found in seeds during reproductive stage, and Se content in the leaves is drastically reduced. For Se non-accumulators such as cereal crops, Se content is the same in grain and roots at maturity stage, with smaller amounts in the stems and leaves (Terry et al. 2000). Selenium non-accumulators contain up to 0.025 mg kg^{-1} (cereals, potatoes, grass, and vegetables), secondary accumulators absorb 0.025 to 0.100 mg kg^{-1} (Aster, Astragalus, Atriplex), and accumulators contain 0.100 to 10 mg kg^{-1} (Stanleya, Haplopappus) (Jezek et al. 2012). The difference in the ability of plants to accumulate Se depends upon the Se metabolism. While non-accumulator species bind Se in proteins, the exclusion of Se from the proteins of accumulator plants is thought to be the basis of their Se tolerance (Neal 1995, Fordyce 2010).

Selenium toxicity in non-accumulating plants results in injury, including stunted growth, chlorosis, withering and drying of leaves, deceased protein synthesis, and premature death of the plant (Mengel and Kirkby 1987). The threshold Se concentration varies with plant age, with younger non-accumulating plants being more susceptible than mature plants (Terry et al. 2000). Moreover, the threshold concentration of Se toxicity for non-accumulators depends on the form of Se accumulated, with selenite being more toxic than selenate. This is due to the faster conversion of selenite to SeCys and SeMet, which are then incorporated into plant proteins in replacement of Cys and Met, resulting in toxicity in the plants (Zayed et al. 1998). The tolerance to Se may increase with increasing sulphate supply (Mikkelson et al. 1989). In this regard, selenate causes higher Se toxicity in plants with low S nutrition. This is associated with an increased ration of Se in proteins versus total Se level, enhanced generation of reactive oxygen species, elevated lipid peroxidation causing increased cell membrane damage, and reduced antioxidant enzyme activities. Selenium toxicity could be counteracted with increased supplementation of S, which decreases non-specific integration of Se into proteins and alters the redox system (Tian et al. 2017). Furthermore, Se induces chlorosis through an adverse effect on the production of porphobilinogen synthetase, an enzyme required for chlorophyll biosynthesis (Padmaja et al. 1989). In addition, in vivo reduction of nitrate in leaves results from interference by excess selenate and selenite (Aslam et al. 1990).

- Selenium in the human body

Selenium is a complex element due to its properties of being both essential and toxic, leaving a narrow range within which intake is healthy. The most important Se exposure route for humans is the diet, as concentrations in food are orders of magnitude greater than in water and air. The estimated average requirements (EARs) are 17 µg day^{-1} for children 1 - 3 years, 23 µg day^{-1} for children 4 - 8 years, 35 µg day^{-1} for children 9 -13 years, and 45 µg day^{-1} for children >14 years and adults (National Academy of Sciences 2000). These requirements were determined based on functional criteria of the minimum Se intake that directly or indirectly reflects the normality of serum Se and glutathione peroxidase-3 (GPX3) activity. This was determined by monitoring changes in the relationship between serum Se and dietary Se supply, and the relatively constant proportionality in the fraction of serum Se to GPx activity (Gu et al. 1998). Globally, serum Se of healthy subjects ranges from 0.52 to 2.50 µmol/l (Alfthan and Neve 1996).

In the diet, organ meats such as liver and kidney are good Se sources and seafood contains almost as much. Muscle meats are also a significant source, while garlic and mushrooms contain more than most other vegetables. Cereals are another important source, but white bread and flour contain about 10 to 30% less Se than whole meal (Fairweather et al. 2011). Selenium content ranges from 0.11 to 0.97 mg kg^{-1} in marine fish, 0.18 to 0.68 mg kg^{-1} in freshwater fish, and 0.08 to 0.73 mg kg^{-1} in meat (WHO 2004). In comparison, Se content in cereal grains ranges from 0.01 to 0.55 mg kg^{-1} (Fairweather et al. 2010). In fruits and vegetables, it ranges from 0.001 to 0.022 µg g^{-1} due to the low protein content coupled with high water content (Fordyce 2007). In general, 79% of Se present in the diet is bioavailable. SeMet is the main compound in plant food sources, particularly cereal crops. Se-methylselenocysteine and γ-glutamyl-Se-methylselenocysteine are found in yeast, garlic, onion, and broccoli. SeCys is the main Se form in animal-source foods (Fordyce 2010). Notably, there is a possibility that losses of nutritional quality of foods may result from cooking (Severi et al. 1997). It has been reported that little or no loss of Se occurs by boiling, baking, or frying meat, seafood, eggs, and cereals. However, dry heating cereals and boiling vegetables result in Se losses (Higgs et al. 1972). Bratakos, et al. (1988) also reported that frying, grilling, and extended boiling could result in some Se loss. In general, the effect of usual cooking procedures results in only minor or no Se losses for most foods (Ferretti and Levander 1974, Dudek et al. 1989, Thompson and Robinson 1990).

Selenium can also become toxic above a relatively low threshold limit. Selenium toxicity in humans is far less widespread than Se deficiency. Toxicity can occur as a result of over-ingestion of Se supplements, ingestion of foods produced on seleniferous soils, and ingestion of foods sourced from areas exposed to industrial selenium pollution. Consumption of high-Se crops grown in seleniferous areas is the main cause of selenosis in humans (Yang et al. 1983). Selenium toxicity is characterized by a higher incidence of gastrointestinal problems (vomiting and diarrhea), disorders of the nervous system, paralysis, poor dental health, deformed nails, dermatitis, and hair loss (Tan 1989). In general, there is a U-shaped association between Se intake and health, i.e.

populations of low Se status benefit from Se supplementation in terms of health outcomes, whereas populations with pre-existing adequate or high status do not benefit but instead suffer detrimental health outcomes (Rayman 2012). At higher levels, Se becomes toxic and non-specific replacement of cysteine by selenocysteine in protein disrupts protein function, causing toxicity and death (El Mehdawi et al., 2011). The tolerable upper intake levels (UL) based on EARs are 90 µg day^{-1} for children 1 - 3 years, 150 µg day^{-1} for children 4 - 8 years, 280 µg day^{-1} for children 9 -13 years, and 400 µg day^{-1} for children >14 years and adults (National Academy of Sciences 2000). However, the upper limit in relation with the toxic effect varies with the geographic region and with population characteristics. For instance, in seleniferous regions in the US, a daily intake of 724 µg day^{-1} has no toxic effects, while in China, selenosis occurs when the daily Se intake is over 910 µg day^{-1} (Preda et al. 2016). Dietary diversification can help to reduce Se toxicity, i.e. high-Se cereal grains can be banned from local consumption in seleniferous regions and instead exported to be mixed with grains from Se-deficient regions.

- Measuring Se status

Many countries across the world carry out national geochemical mapping programs based on systematic collection of soil, sediment, water, rock, and vegetation. These materials are analyzed for a range of element compositions and used to produce maps of element distribution in the environment (Fordyce 2010). Selenium is however difficult to analyze partly because concentrations in natural materials are very low, and its analytical techniques are more expensive compared to those of other compounds monitored in routine analytical programs. As a result, Se is often excluded from the group of determinants despite its importance. Although there is equipment like Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) that is able to detect trace levels of Se, this has a high acquisition and maintenance cost that makes Se analysis very expensive. Furthermore, specialized staff and technical support are needed to successfully operate the equipment, as well as specific instrumental settings and sample preparation procedures, not allowing Se to be determined in a same run as other minerals (Fairweather et al. 2011, Bueno et al. 2007). This likely explains why Se deficiency has in the past been omitted from national nutrition surveys in Kenya, and hence excluded from past interventions. Furthermore, local food composition tables (FCTs) are incomplete with missing local foodstuffs and some nutrient contents including Se concentration. As a result, local nutrition researchers and dietitians are compelled to borrow values from foreign FCTs leading to poor nutritional decisions. Lack of comprehensive local FCTs limits the reliability of research or diet planning results (Chege and Ndungu 2016, Murphya et al. 2004, Willliamson 2005).

The Se content of foods varies based on the soil's soluble Se content and the capacity of the crops to take it up. As a consequence, various foods show geographic patterns of variation in Se content, which reflects local soil Se conditions at the points of origin. Selenium intake of individuals or populations is assessed by the product of Se content in food and amounts of the foodstuffs consumed. It is not a requirement to have a high Se absorption rate to be able to use total dietary

Se intake as a predictor for Se status, on the condition that the estimated dietary requirements to be compared are also based on population studies relating human Se status to total dietary Se intake. Such assessment of nutrient status is usually done using standard food composition tables combined with assessment of food intake. However, this yields inaccurate estimates of Se intakes because geographic variation in food Se contents is not captured in the food composition tables. Determination of food Se content by actual analysis of a wide range of representative samples is necessary to produce a valid estimate of Se intake. Estimates of total dietary Se intake are sufficient to determine whether individuals and populations meet the recommended Se intakes (Combs et al. 2015). Single-day dietary surveys generate errors when being used to estimate the real long-term exposure to Se, because of the wide variation in daily intake. Comparisons of different methods have shown that 3-weeks dietary observations provide good estimates of overall intake and consist therefore of a much more reliable indication of the Se status (WHO, 1987). Dietary data based on the 1-day 24-hour recall (24HR) method (Willett 2013), conducted on a random day in order to include all days of the week, deliver reliable estimates of the average usual intake of a population group. However, the data cannot be used to estimate distribution of the intake (Council National Research 1984).

Due to errors in standard food tables, it would be useful to impute Se intake from measured biomarkers of Se status. This approach is most straightforward in subjects of deficient to low Se status, whose plasma Se concentration responds to supplemental Se in proportion to the magnitude of supplementation (Combs et al. 2015). Various bio-indicators of Se status are used to assess Se status. Most absorbed Se is taken up by the liver and re-enters the blood circulation as a component of selenoprotein-1 (SEPP1), which is the primary transporter of Se to peripheral tissues (Burk and Hill 2009).

Estimation of retained Se is inferred from analysis of SEPP1, GPX3, and the non-specific component of plasma Se. These serve as an indication of the degree to which an individual has met his/her Se requirement and may have Se reserves. These selenoproteins show maximal expression at a plasma Se concentration of 50 to 70 ng ml^{-1} (Clausen and Nielsen 1998). Plasma (or serum) Se is commonly used as it responds to an increase or decrease in Se intake. However, plasma Se concentrations are subject to metabolic demands, e.g. during illness, that lower the concentration as a result of redistribution rather than of a nutritional deficit (Sattar et al. 1997). Therefore, functional Se deficiency is instead assessed by measuring selenoproteins and particularly SEPP1 and GPX3 in whole blood and red blood cells. Whole blood and red blood cell Se concentrations do not vary during metabolic demand and therefore, they are also used as an indicator of deficiency. SEPP1 and GPX3 are useful under conditions of Se intake within the range of sub-optimal Se intake that necessitates regulation of selenoprotein expression i.e. <55 µg day^{-1} (Combs et al. 2015). GPX3 is therefore a useful biomarker in people with relatively low status (Ne`ve 1991) and is extensively used to measure Se status due to its close association with dietary Se intake. The enzyme activity represents functional Se and its assessment is easier to perform

than Se analysis. The method however requires caution because GPX3 activity is influenced by other physiological factors and non-Se-dependent GPX3 is also present. However, GPX3 activity also reaches a plateau with increasing Se intake and so it is not a good indicator of toxicity (WHO 1996). Red blood cell Se concentration reflects longer-term status due to the incorporation of Se during the cell synthesis (Katz and Chatt 1994). However, since the red blood cells take around four weeks to respond to changes in intake, they are unsuitable for assessing acute toxicity.

Renal excretion is the main means of Se homeostasis in humans and therefore urine Se concentrations are usually dependent on intake, and urine Se measurement is considered useful for monitoring Se exposure (Ashton et al. 2009). Urinary Se excretion is related to Se status (Jackson et al. 2013) because a significant amount of circulatory Se (15 to 20%) is released in the urine over several days. Selenium retention in the body can therefore be assessed by the difference between the amount of Se ingested and the amount excreted. Excess absorbed Se is eliminated in the urine in form of methylated products, which increases with increased Se intake (Combs et al. 2011). Urine Se concentration outside the normal range may indicate deficiency or toxicity, although urine measurements are more variable and more difficult to interpret in isolation compared to plasma measurements.

Hair and toenails are used to assess long-term Se status and offer the advantage of simple and low-cost sample storage. Their analysis requires considering whether subjects used Se sulfide-containing anti-dandruff shampoos. With standardized procedures for collecting hair or toenail samples, Se levels correlate well with blood/plasma Se concentration (Yang et al. 1989). Their use to assess Se status relies on the assumption that their Se content reflects some metabolically relevant component of body Se, an assumption that has never been validated. Hair/nail Se is an excretory form of the element, and hence represents portions of Se that were excreted in the past, reflecting Se status at that time. Such data are useful for populations with stable dietary practices. Hair/nail Se contents therefore indicate the likelihood of deficiency or adverse effects, but they do not provide direct evidence of either condition. Their value lies in providing information about Se status over a wide range of food Se intake levels (Combs et al. 2015).

1.1.2. Se deficiency – an overview

- Prevalence of dietary Se deficiency

Globally, approximately one billion people are affected by Se deficiency (Combs 2001). Dietary Se intakes vary hugely both between countries and within countries (Parr et al. 1992). For example, in China, Se intake for adults ranges from 3 µg day^{-1} in the Keshan disease area to 1338 µg day^{-1} in the seleniferous areas. In European countries, the estimated dietary Se intake ranges between 30 and 50 µg day^{-1} (Fairweather-Taint et al. 2011). Estimates of dietary Se intakes and status are scarce for Africa. However, recent studies in the region indicate that the risk of Se deficiency is widespread. Chilimba et al. (2011) reported widespread suboptimal dietary Se intakes in Malawi, which was attributed to low levels of plant-available Se in most soils and narrow food choices.

Inadequate dietary Se intakes of 20 to 30 µg day^{-1} were reported based on national consumption patterns. Hurst et al. (2013) reported Zambia at the highest risk of dietary Se inadequacy at 91 - 100%, followed by Zimbabwe, Malawi, Burundi, and Democratic Republic of Congo at 76-90%, and Central Africa, Kenya, Ethiopia, and Mozambique at 26 - 75%. Joy et al. (2014) further reported Kenya, Burundi, and Liberia at the highest risk of dietary Se deficiency, i.e. 91 - 100%. Overall, the estimated dietary Se intake in Africa ranges from 23 to 35 µg day^{-1} with a mean estimated risk of Se deficiency of 28%, which was more widespread in the East African region at 52%. In Malawi, Hurst et al. (2013) further showed that Se deficiency was likely to be endemic based on the Se status of adults consuming foods from contrasting soil types. Over 80% of the Malawi population was reported at risk of dietary Se inadequacy. In addition, Joy et al. (2015) reported that >50% of households in Malawi were at risk of Se deficiency due to inadequate dietary supplies. More than 80% of the rural households living on low-pH soils had inadequate dietary Se supplies compared to 55% on calcareous soils. Additional studies in Africa have in the past reported high Se deficiency including the study of Amare et al. (2012) in Ethiopia, who reported Se deficiency among school children to be higher than Zn deficiency at 62% and 47% respectively, while Gashu et al. (2016) reported associations between selenium deficiency, stunting, and anemia with poor cognitive performance in preschool children from rural Ethiopia. However, these observations are associations and hence, the specific causality of poor cognitive performance cannot be ascertained. In Zimbabwe, Se deficiency was estimated at 48 % among school children (Kuona et al. 2014). Baeten et al. (2001) associated Se deficiency with higher likelihood of genital mucosal shedding of HIV-1 infected cells, increasing the infectiousness of women with HIV-1 in Kenya. A study in Sahelian area of Niger reported very low plasma Se levels of <25 ng ml^{-1}, which are comparable to those noted in epidemiologic studies on the Keshan disease. Peripartum cardiomyopathy is in fact a very common disease in this region (Cena et al. 1992). Despite the widespread Se deficiency in SSA and subsequent negative implications on health, it is the least studied deficiency in the region. There is therefore a need to assess the actual risk of Se deficiency and its impact on health of the affected communities in several SSA countries, and develop suitable intervention measures.

- Etiology of Se deficiency

Micronutrient deficiencies in Kenya have a multifactorial etiology that includes among others, persistent food insecurity, parasites, and infectious diseases (Fanzo 2012). Food insecurity in particular is mainly contributed by a widespread poverty (Townsend 2015). About half of the Kenyan population, mainly in rural areas, is unable to afford sufficient and diversified diets to meet recommended daily requirements. The poverty trend indicates a stagnant aggregate on average welfare gains for the poorest quintile (World Bank 2009). In addition, corruption, climate change, large family size, and illiteracy contribute to poor nutrition (Bain et al. 2013).

Low diverse diets based on a few staple foods that supply energy and low amounts of micronutrients are recognized as a potential key factor to micronutrient deficiency in SSA (Kennedy

et al. 2007). Access to high-quality nutritious foods remains a major challenge among rural populations (Fanzo 2012). In Kenya, agriculture is rain-fed and supports a few crops, mainly maize and beans. Rural households therefore depend on these grains all year round. Over the years, the inheritance of land across generations has resulted in households depending on about 1 acres of land for food production. The households therefore practice continuous cropping under usually poor soil management practices, which depletes soil's nutrients and leads to low food production. In addition, high food prices and low purchasing power limit rural households from purchasing a variety of foods and especially good dietary Se sources such as animal-source foods. Although Kenya is a relatively large country (acreage is equal to 143.412 million), arable land covers only 18% of the land surface, with the rest consisting of forests and marginal lands (Sombroek et al. 1982). Food production in the past has focused on quantity rather than quality. The high dietary Se deficiency in rural Kenya can therefore be attributed to inadequate food production and is exacerbated by a low diversified diet (Kennedy et al. 2007, Townsend 2015). In addition, the Se status of agricultural soils and intrinsic soil geochemical factors that inhibit Se mobility and availability for plant uptake affect its concentration in the crops and animals grown on these soils (Dhillon and Dhillon 1999, Gibson and Hotz 2002, De Temmerman et al. 2014). Inadequate dietary Se intakes can therefore be influenced by environmental factors that affect the fate and mobility of Se in the soil (Du Laing et al. 2009).

Globally, total Se concentration in soils lays within the range 0.01 to 2.0 mg kg^{-1} with a mean of 0.4 mg kg^{-1} (Rayman 2008). The Se deficiency threshold in agricultural soils more specifically ranges from 0.1 to 0.6 mg kg^{-1} (Fordyce 2013). In China, a geochemical analysis of areas with high incidence of Keshan disease showed that total soil selenium was not inversely correlated with Keshan disease incidence, as expected, but Keshan disease incidence was, however, inversely correlated with water soluble soil selenium (Fairweather-Tait et al. 2011).

Beside diet and soil composition, awareness of the population about micronutrient deficiency is hypothesized as another important aspect to the design of suitable solutions. Indeed, a disorder like Se deficiency often has no visible warning signs, thus earning the name "hidden hunger". The affected population is largely unaware of its detrimental health effects (Biesalski 2013). In Kenya, the prevalence of Se deficiency remains unknown. Societal awareness of the regional distribution of Se deficiency risks and of subsequent health implications is fundamental in resolving it.

The issue of Se deficiency addressed in this thesis and associated research questions with respect to the design of potential solutions to resolve it are therefore highly relevant and timely. This thesis assesses the Se deficiency status of a region and population in Kenya and tests how the factors highlighted above contribute to explaining this status in the setting considered. This primary stage of research lays the basis to further investigate the effectiveness of possible intervention measures.

- Selenium and human health

In recent years, Se research has attracted research interest because of its important role in antioxidant selenoproteins for protection against oxidative stress initiated by excess reactive oxygen species (ROS) and reactive nitrogen species (Tinggi 2007). In the body, Se has beneficial health properties due to its antioxidant capacity through cellular protection from free radicals (Nogales et al. 2013). The biological effectiveness of Se is based upon the integration of selenocysteine into the active center of 25 selenoproteins with biological properties. Glutathione peroxidase (GPx) for instance is a major selenoprotein involved in regulating oxidative processes and cell membrane protection (Papp et al. 2007) by catalyzing the reduction of hydroperoxides and lipid peroxides to their corresponding alcohols and water with reduced glutathione (GSH) as the electron donor (Brigelius-Flohe and Maiorino 2013).

Inadequate Se intake levels below 20 µg day^{-1} result in clinical deficiency disorders, and intakes less than those needed for maximal expression of glutathione peroxidase (i.e. 40 µg day^{-1}) increase the risk of health disorders (Fairweather et al. 2011). The increased production of ROS due to Se deficiency exerts oxidative stress in the physiological system, and if they are not properly regulated, they can cause damage to cellular lipids, proteins, and DNA. This damage has been linked to the pathology of heart disease i.e. oxidation of low-density lipoprotein (LDL), which is associated with initiation of atherosclerosis in heart disease. One hypothesis is that the presence of high Se as antioxidant selenoenzymes and selenoproteins may help to reduce the production of oxidized LDL, and hence would reduce the incidence of heart disease (Furman et al. 2004). Growing evidence also suggests an association between Se and the processes that lead to or prevent cancers (Gather 1999). There have been numerous animal studies indicating the important role of Se in reducing and preventing the incidence of cancer initiated by a both chemical and radiation carcinogens. In addition, evidence from human epidemiological studies has increasingly indicated an inverse relationship between Se status and cancer risk in human populations (Clark et al. 1996). Even though Se is reported to play a significant role in cancer development, its exact anticancer mechanism of action at molecular level is not fully understood (Tinggi 2007). However, it has been hypothesized that the most possible mechanistic action of Se as a chemopreventive agent is its role in the antioxidant defense systems to reduce oxidative stress and limit DNA damage (Rayman 2005). In addition, Se deficiency has been associated with decreased survival in HIV-infected patients (Rayman 2000). However, associations between low serum Se and low CD4+ cell count or high viral load could be due to the lowering of blood Se concentration as a result of the increased metabolic demands in individuals with more advanced HIV-infection (Drain et al. 2006). Besides, Se deficiency has been linked to the incidence, virulence, or disease progression of other viral infections (Rayman 2000, Broome et al. 2004). The pathogenesis of edema and anaemia found in children with protein-energy malnutrition has also been suggested to be caused by an imbalance between the production of toxic radicals and their safe disposal, although this hypothesis is yet to be confirmed. It is suggested that children with protein-energy malnutrition are potentially

susceptible to high oxidative stress (Ashour et al. 1999). It is further suggested that Se regulates somatic growth during early life by protecting cell membranes from oxidation that could disrupt tissue metabolism and hence growth. This is a crucial role since oxidative stress is responsible for impaired intrauterine growth retardation and hence, inadequate maternal intakes can result in stunting due to changes in the foetus growth and length at birth (Mistry et al. 2012). Selenium deficiency has also been associated with occurrence or persistence of endemic goiter, however, these observations are associations and hence, the causality cannot be ascertained (Kishosha et al. 1999, Vanderpas et al. 1990). Generally compared to common mineral deficiencies in sub-Saharan Africa (SSA) such as zinc (Zn), iodine (I) and iron (Fe), Se deficiency is the least studied, and therefore not considered as an important public health problem. This is despite the fact that Se has multiple functions in the body.

1.1.3. Approaches for addressing Se deficiency

- Interventional and policy measures

Addressing micronutrient deficiencies to reduce health-related issues can be sustained or enhanced through various types of interventions. These include diet diversification, commercial fortification of processed foods, nutrient supplementation, and biofortification (Lawrence 2005). In Kenya, interventional measures used to solve micronutrient deficiencies include fortified commercial foods and supplementation (Micronutrient initiative 2009, Ministry of Health Kenya 2013). Food fortification is included in the national food security and nutrition policy under which the Kenyan legislation on food fortification requires the table salt to be fortified with iodine to a standard level of 50 to 84 mg kg^{-1}, wheat flour to be fortified to a standard level with zinc (40 to 80 mg kg^{-1}) and iron (not less than 20 mg kg^{-1}), and maize meal to be fortified to a standard level with vitamin A (0.5 to 1.4 mg kg^{-1}), zinc (33 to 65 mg kg^{-1}) and iron (21 to 41 mg kg^{-1}) (MoH 2018). Existing supplementation programs administered by the ministry of health on the other hand include vitamin A supplementation for mothers and children, and iron and folic acid supplementation for pregnant women (Ministry of Health Kenya, 2013, Fiedler et al. 2014).

In practice, rural populations have limited access to these commercial fortified foods and nutritional supplements. More feasible population-based strategies are needed to complement them to reach these communities. Agronomic biofortification is a strategy that supports mineral-dense crops in rural areas where soils have insufficient concentrations of mineral elements required for human nutrition (Lyons et al. 2004a). It aims at improving nutritional content of staple foods consumed by the affected population (Layu et al. 2013). The strategy has proven to be effective for Zn, Se, and I, as tested in Turkey and China (Cakmak 2014, Jiang et al. 1997). An important consideration in designing suitable interventional measures is the fact that no single hidden hunger can be addressed in isolation because it exists together with other forms of malnutrition (Bouis et al. 2011). This calls for the need to combine different population-based strategies including public awareness, diet diversification, and even multi-nutrient biofortification intervention (Valença et al. 2017). Up to

now, no intervention measure is in place in Kenya for Se deficiency and its health implications in such contexts, where existing intervention strategies and policies do not apply. This thesis contributes to filling this gap by evaluating the effects of agronomic biofortification in improving dietary Se intake in rural households, and ultimately in reducing the risk of Se deficiency.

- Agronomic biofortification intervention

Agronomic biofortification consists of applying fertilizers of mineral elements lacking in the diet in order to increase their concentrations in food (Lyons et al. 2004b, Lavu et al. 2013), through soil or foliar Se fertilizer applications (Ros et al. 2016). The strategy targets important staple foods consumed by low-income households (Lyons et al. 2004a). Agronomic biofortification can contribute to lower rates of malnourished population, and to contribute to maintaining improved individuals' nutritional status (Bouis et al. 2011). Through this strategy, biofortified foods first reach remote populations comprising a majority of the malnourished, and then penetrate to urban populations as production surpluses reach the urban market (Bouis and Saltzman 2017). Selenium fertilization on crops has proven to increase Se concentration in staple grains and subsequently dietary Se intake. This was observed for instance in a successful national interventional case study in Finland where nationwide Se biofortification for the last 30 years has resulted in the Se content in cereal crops being increased by 15-folds (Alfthan et al. 2015).

This thesis explores the effect of soil and foliar Se fertilization on Se concentration in staple grains. Based on a comparison of their effectiveness in improving dietary Se intake, the thesis then selects the most suitable Se fertilization dose and application technique that can potentially improve dietary Se intake in locations of high Se deficiency risk in Kenya.

1.2. Research objectives and hypothesis

Based on the above background, the research objectives of this study are to:

i. Investigate the Se status, dietary Se intake, and risk of Se deficiency for the Kenyan population
ii. Map the Se deficiency distribution and identify locations at high risk of dietary Se deficiency
iii. Assess the staple crop's response to Se fertilization doses and techniques, and determine a suitable Se biofortification intervention strategy
iv. Evaluate the impact of the Se biofortification intervention on the risk of dietary Se deficiency

Based on the achievement of the above objectives, the study hypothesizes that agronomic biofortification of staple grain crops can help to abate the risk of dietary Se deficiency among the affected rural communities in Kenya.

1.3. Contributions of the research

The thesis **first** contributes to the literature by being the first study to provide Se status data of the Kenyan population. Based on EARs, inadequacy of dietary Se intake is used as evidence of the risk for Se deficiency and hence the need for an intervention to address this silent epidemic. Such a comprehensive analysis is necessary to develop a suitable Se-deficiency intervention for rural subsistence farming settings.

Second, the thesis provides an understanding of the key contributing factors to the risk of dietary Se deficiency. This includes an understanding of the effect of soils' geochemical characteristics on Se status of local agricultural soils and Se mobility and availability for crops' uptake. This is a necessary step to develop potential agronomic intervention measures. Rural households depend on small acreage of land for staple foods production. Micronutrient deficiencies result from continuous cropping without nutrient replenishment and over dependence on mineral deficient staple foods. Thus, the thesis elucidates the relationships between the soil geochemical characteristics in different agricultural soils and Se concentration in local foodstuffs, and between dietary Se intake and the population's Se status. The thesis further divulges variation in risk of dietary Se deficiency caused by climatic, environmental, cultural, and dietary patterns. Based on the collection of this wide range of primary data, this research enables to identify contributing factors of Se deficiency from the soil, diet, and to the body. This is highly relevant to the design of interventional measures such as the agronomic biofortification as studied in this thesis, and associated policies.

Third, through measuring the average dietary Se intake of the population, the thesis contributes to filling the gap of representative Se intake data in developing countries (Fairweather et al. 2011). More specifically, it provides the missing mineral concentration data currently unavailable in the local FCTs, in addition to average dietary intake data. Laboratory analysis data for actual mineral concentration will indeed be used to update local FCTs. Making this data available is important to diminish reliance on foreign FCTs that often result in misleading results. The shortcoming of borrowing nutrient content data from foreign FCTs is due to regional variance in mineral concentration in foods influenced by differences in soil geochemical status, season, crops' growth rate, animal or plant species, agricultural practices, and food processing or preparation. This variation is more problematic for micronutrients (vitamins and minerals) than macronutrients (Kaminski and Christiaensen 2014). To avoid estimation errors, availability of actual mineral concentration data and dietary intake data is obligatory to ensure reliability of research or diet planning results (Chege and Ndungu 2016). Thus, the thesis combines average dietary intake data with Se concentration of local foodstuffs in order to calculate the average dietary Se intake.

Fourth, based on the hypothesis along which agronomic biofortification strategy is an efficient and feasible option to increase Se concentration in staple foods, the thesis contributes to the understanding of local crop's response to Se fertilization in a set of locations that differ in agricultural

soils and climatic or environmental conditions. The thesis recognizes the lack of standard and sustainable agricultural practices in rural subsistence farming that results in mineral-deficient soils. It therefore contributes to the literature by evaluating the effect of replenishing soil P and N on Se concentration in staple grains. In addition, the study contributes to the evaluation of the effect of combining Se fertilizer with other mineral fertilizers such as Zn and I for a possible simultaneous multi-mineral intervention. The thesis highlights the most effective options among different biofortification strategies, and recommends the most suitable Se biofortification doses and techniques depending on the Se deficiency risk level of a region.

Fifth, using a cluster-randomized control trial, the thesis contributes to the literature on the impact of Se agronomic biofortification with average dietary Se intake being the outcome measure of Se status of the population. This is tested with a foliar Se fertilization at 20 g Se ha^{-1} on maize crops, which was found to positively and significantly impact Se concentration in maize grains, dietary Se intake from maize, average daily dietary Se intake, and ultimately the reduction in Se deficiency risk. The net impact of the intervention is measured, accounting for the potential influence of Se concentration in soils that may influence Se uptake by crops. The thesis therefore provides a unique evidence of the potential of a population food-based approach in addressing Se deficiency in limited resource settings.

1.4. Outline of the research

The remainder of this thesis is composed of six chapters. **Chapter 2** presents the main study region - Central Kenya Highlands - and the study population groups. The chapter then explains the chemical elemental analysis and other methods that are commonly applied in subsequent chapters.

Chapter 3 presents the results of a cross-sectional survey. It provides an overview of Se deficiency status in the Central Kenya Highlands, which allows identifying locations at high risk of dietary Se deficiency. It also points out the factors influencing Se deficiency risk across the study locations. Based on the inadequacy of dietary Se intake, the chapter highlights the need to develop interventional measures to tackle the hidden hunger.

Drawing from Chapter 3, **Chapter 4** evaluates the associations between indicators of dietary diversity and dietary Se intake in rural Kenya. Specifically, it explores the nature of the diet in terms of composition and variation and their contribution to explain the risk of dietary Se deficiency. Thus, the chapter presents the results of an in-depth analysis of the impact of food composition and diet diversification on the risk of dietary Se deficiency based on different regional contexts influencing the dietary pattern.

Chapters 5 and 6 address measures that ultimately aim at improving dietary Se intake and hence reduce the risk of Se deficiency in affected communities. The two chapters evaluate the impact of Se biofortification on Se concentration in staple grains. **Chapter 5** in particular assesses the

response of maize and bean crops to various Se fertilization doses and techniques, and the effect of combining Se fertilizer with other mineral fertilizers such as P, N, Zn, and I, on Se concentration in grains. The chapter concludes by identifying the most suitable Se fertilizer application dose and techniques and the possibility of a simultaneous multi-mineral biofortification intervention. **Chapter 6** builds up from the results of Chapter 5 and presents a cluster-randomized controlled intervention trial based on a foliar Se fertilizer application dose of 20 g Se ha^{-1} on maize crops in a location at risk of dietary Se deficiency. The impact of the biofortification intervention on dietary Se deficiency risk is assessed through a comparison between a control group and an intervention group at baseline and post-trial.

Finally, **Chapter 7** concludes and discusses the results of the thesis and the limitations of this research.

CHAPTER 2: METHODS

2.1. Study design

2.1.1. Study regions and population

The research was conducted in a semi-arid and high-rainfall area in Kenya, where agriculture covers about 18% of the land surface of the country, with the rest consisting of forests and marginal lands. The study areas are located in agro-climatic zones that are home to 74% of Kenya's population (Sombroek et al. 1982). These zones have the highest volume of staple food production, ethnic diversity, and the largest proportion of malnourished children (Gachene and Kimaru 2003). The thesis covers the Central Kenya Highlands and Lake Victoria Basin for comparison purposes. Indeed, fish from natural sources is one of the best dietary Se sources. While fish intake is expected to be high in the Lake Basin, it is rarely consumed in the Central Highlands. Study locations in each of the regions are selected based on regional soil maps such that each location represents a different agricultural soil type. Those are presented in detail in subsequent chapters. Agricultural soil type is indeed hypothesized in a context of rural subsidence farming, as a key factor contributing to dietary Se deficiency. This is because rural households depend on monotonous diets based on mainly maize and bean grains, and sometimes leafy vegetables and potatoes are added when in season. Kenya has different agricultural soils types owing to variation in geology, relief and climate. The major soils in the target agro-climatic zones include ferralsols, acrisols, vertisols, lixisols, luvisols and nitisols (Gachene and Kimaru 2003). Table 1 shows the general characteristics of the soils primarily occurring in the study locations (Jaetzold et al. 2006), while Figure 2 shows a generalized soil map of Kenya, indicating the location of the two studied regions.

Table 1: Broad estimate of the dominant characteristics of the soils primarily occurring in the study locations (based on International FAO classification)

Major soil class, unit	Texture subsoil	Texture topsoil	Depth	[1]OM of topsoil	pH (water)	Drainage	Fertility
Acrisols	clay	variable	variable	variable	<5.5	moderate to good	low
Andosols	clay	clay	deep	moderate to high	variable	good	high
Arenosols	sand	sand	variable	low	variable	good	wow to very low
Cambisols	variable	variable	variable	variable	variable	moderate to good	moderate to high
Ferralsols	clay	clay	variable	variable	variable	good	low
Fluvisols	variable	variable	variable	variable	variable	variable	moderate to high
Histosols	peat-clay	peat	variable	very high	<5.5	moderate to poor	moderate to high
Lithosols	rock	variable	very shallow	variable	variable	variable	variable
Luvisols	clay	variable	variable	variable	>5.5	moderate	low to moderate
Nitosols	clay	clay	deep	moderate - high	variable	good	moderate to high
Phaeozems	clay	variable	variable	high	>5.5	good	high
Planosols	clay	variable	variable	low to moderate	variable	poor	low to moderate
Regosols	clay	variable	variable	moderate to high	variable	variable	variable
Vertisols	clay	clay	variable	low to moderate	variable	poor	moderate to high

[1]OM: Organic matter content. [2]WHC: Water holding capacity

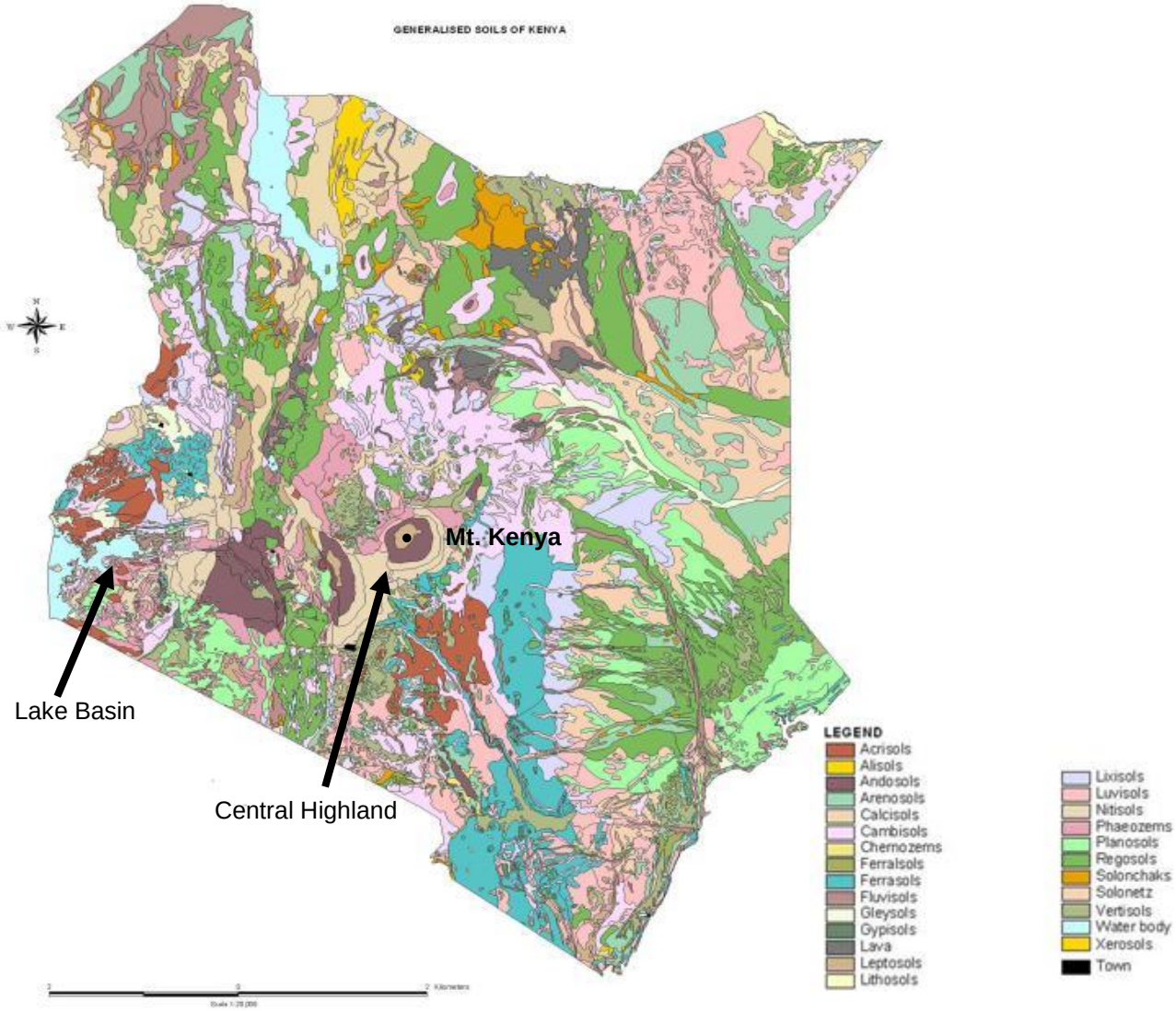

Figure 2: Generalized soil map of Kenya (*Source: Kenya Soil Survey*), indicating the location of the two studied regions.

Due to the role that Se plays in growth and development, the importance of maternal nutrition during pregnancy and lactation, and vulnerability of children and women to micronutrient deficiencies (Darnton-Hill et al. 2005), children and women in rural areas within reproductive age were the targeted population for this research. The thesis investigates the risk of dietary Se deficiency among children aged 6 to 59 months and women aged 19 to 39 years in rural areas of the study locations in the two regions. Excluded from participation were women and children with congenital or chronic abnormalities impairing feeding pattern, severely ill, with clinical conditions under feeding regimes deviating from a regular diet, or non-permanent residents of the study area. All subjects were informed about the study objectives, procedures and measurement methods, and signed a consent form. The survey was approved and cleared by Kenyatta National Hospital Ethics & Research Committee (KNH/UoN - ERC) - Nairobi, Kenya, and University Hospital (UZ) Medical Ethics Committee of Ghent University, Belgium. The biofortification intervention trial was instead

approved and cleared by Meru University of Science & Technology Institutional Research Ethics Review Committee (MIRERC) in Kenya, and was registered in the Pan African Clinical Trials Registry (PACTR) with trial number PACTR201710002698231.

2.1.2. Research design overview

The overall research design is summarized in Figure 3. A cross-sectional survey first assessed Se status in the Central Kenya Highlands and the Lake Basin. The Se agronomic biofortification experiment was subsequently set up in locations identified at high risk of dietary Se deficiency in order to investigate the response of staple crops to alternative Se fertilization doses and techniques. A cluster-randomized controlled trial then evaluated the effect of Se biofortification intervention on the risk of dietary Se deficiency.

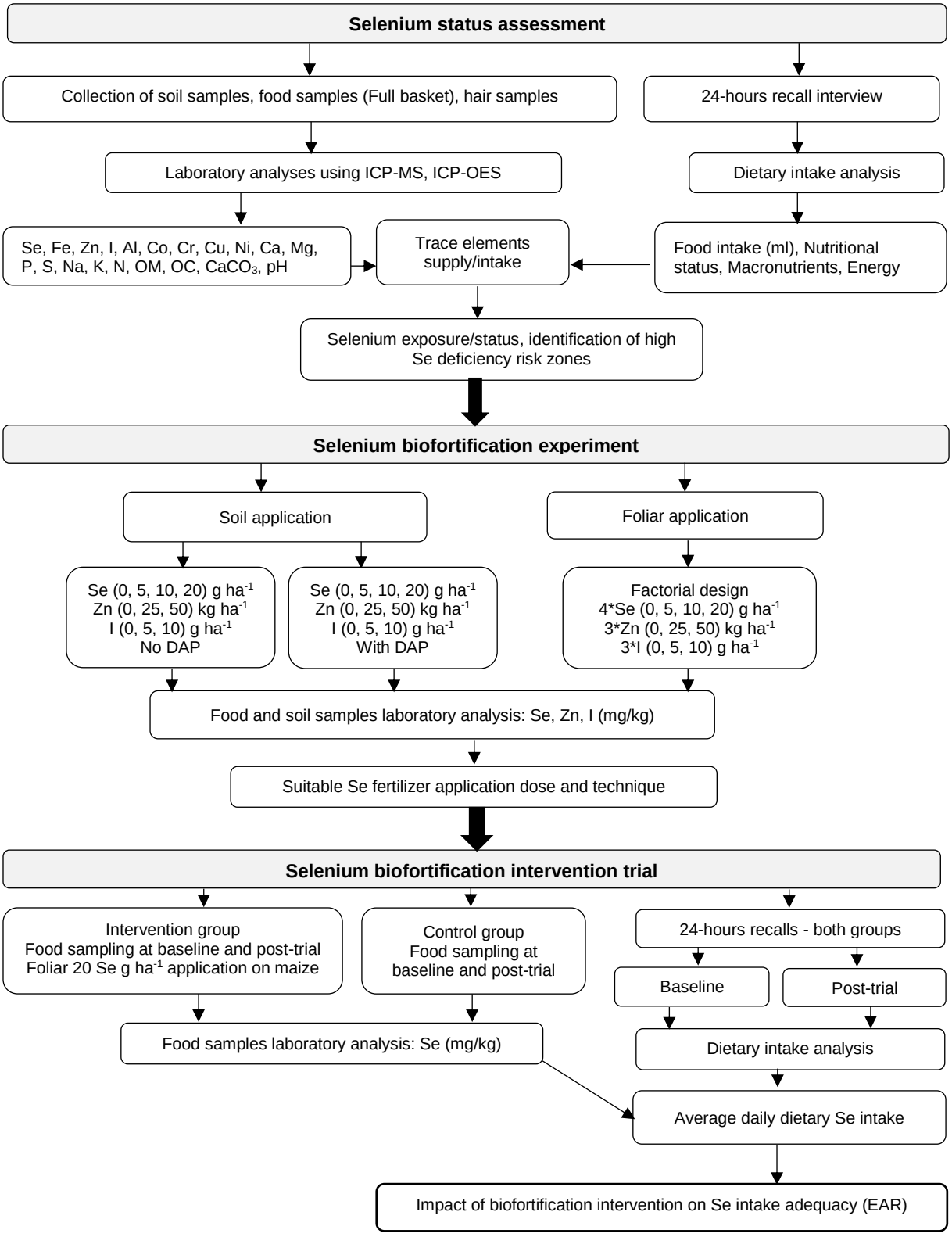

Figure 3: Study design

2.2. Assessment of selenium intake and status

Dietary intake assessment was conducted to assess Se status in the population. This implies the estimation of average dietary Se intake, based on estimated mean food intake amounts and Se concentrations of the foods.

2.2.1. Food intake amount estimation

Food intake amount was estimated using the 1-day 24-hour recall (24HR) method (Willett 2013). The dietary intake assessment was conducted by trained nutritionists on women and mothers/guardian on behalf of their children, on a random day in order to include all days of the week. This comprehensively captured different meal mixtures prepared by the households throughout the week and hence the diversity of foods available at the time of the study. This type of dietary data can be used to obtain reliable estimates of the average intake of a population group as needed for the current study. However, the data cannot be used to estimate the intake in individuals nor provide information on an individual's food intake variability. To achieve this objective, multiple non-consecutive 24-hour recalls on the same individual are instead required in order to capture daily variability (Council National Research 1984). A major limitation of this method is the inability to measure the impact of seasonal variation unless conducted on different occasion during the year.

Respondents were issued with recall kits a day before the interview to practice estimating portions of food consumed, and were asked to only report the actual food eaten (included any food/drink added and subtracted any leftovers). A standard protocol (multiple pass approach) was applied, which consisted of five steps:

1. List: a list of foods and beverages consumed during the previous 24 hours was collected
2. Forgotten foods: probed for foods possibly forgotten during step 1
3. Time and occasion: time and occasion for each food was collected
4. Detail cycle: detailed description, amount (ml), and additions or remains for each food was collected
5. Final probe: probed for anything else consumed in the previous 24 hour

Due to lack of standard serving portion sizes, respondents estimated the volume of foods and drinks consumed using the plate or cup used for feeding. They estimated the amounts consumed using rice grains to represent any foods or drinks consumed. The rice was then transferred into a calibrated jar to measure the volume (ml). It should be noted that the use of rice grains did not mean respondents consumed rice nor represented an ingredient for cooking. The rice only aided in demonstrating the estimated amounts of foods and drinks consumed. In addition, respondents provided recipes which were mainly a mixture of cereals and legumes, and sometimes addition of tubers and leafy vegetables. Drinks mainly included white tea, milk and porridge. Based on the ratio

of ingredients, the volume of each food consumed was re-computed, and confirmed by the respondent during the interview. Through practical food preparation based on the recipes provided, the change in food volume due to cooking was determined i.e. on average, 500 ml water and 350 ml maize flour = 700 ml ugali, 500 ml grains = 750 ml flour and 402 ml raw rice = 720 ml cooked rice. Since raw foods were analysed for Se concentration and not the cooked foods, the change in volume due to cooking was corrected for i.e. an average correction factor of 0.53 was used for cereal grains. Amounts of foods consumed (g FW) were computed based on the mean moisture content of foods (Supplementary information 1). The estimated dietary Se intake was then calculated as the product of the amount of foodstuff consumed (g FW) by a participant and the Se concentration in the food (mg kg^{-1} FW) sampled in his/her farm/household. The average dietary Se intake was compared with EARs in order to assess dietary Se intake adequacy.

During the same round of interview, participants' anthropometric parameters were measured by the same trained nutritionists. Tared weighing was conducted using the UNICEF Seca scale 878. For height and length measurements, a Seca 213 portable stadiometer was used for women and a length-board for children. The weight and height measurements were repeated twice, and for any discrepancy between the first and the second measurements of above 0.2 kg for the weight and 0.1 cm for the height, a third measurement was done. Weight was recorded to the nearest 0.01 kg while height/length to the nearest 0.1 cm. WHO Anthro Survey Analyser was used to analyse the z-scores and the WHO global growth reference of 2006 used as reference Z-scores for the weight-for-age (WAZ), height-for-age (HAZ), and weight-for-height (WHZ) for children (WHO 2006). Body mass index for women was calculated as weight (kg)/height (m^2).

2.2.2. Sampling procedure

Food was sampled from the fields belonging to the households selected to participate in the study. The sampling of the households which differs depending on the goal of the subsequent chapters, is described per chapter. If the food was already harvested, available food stock was sampled. Notably, any food available in the household was sampled including food sourced from the local market. Since this study aimed at understanding Se supply in the diet, the analysis of all types of food consumed by the population irrespective of the source was therefore necessary. Notably, subsistence farmers sell their grains to local retail traders as a source of income or to avoid postharvest loses. Overtime, they re-purchase the grains from the same market. The cereals in the market are therefore a mixture of grains from the surrounding villages. In this study, each study location presented an agricultural soil type of which, all the selected villages were within the target soil type. Since the rural trading centres serve villages within a radius of ~10 km, which is within the area studied per location, the cereal grains in the market are therefore cultivated within the target soil type. During the survey, retail traders in the market confirmed that their foodstuffs were supplied from local farms. Food sampled included cereals, legumes, tubers, leafy vegetables, fruits, milk, meat, fish, and eggs. To sample grain-based foodstuffs, the grains were spread on a mat,

mixed together and a composite sample was taken. Cereal and legume grains were winnowed to remove chaff, tuber crops washed, peeled and the edible part cut into pieces, while leafy vegetables were cleaned to remove dirt, dead parts, and foreign plants. To sample animal food products such as meat, milk, and eggs, samples were sourced from different shops to ensure a representative sample. In the case of meat, the butcher was asked to sample different parts of the carcass in order to ensure a representative sample for the different body parts. Organ meats were sampled in the same manner but as a separate sample. Volume (ml) of raw food samples was measured in calibrated measuring cups and weighed (g) before drying for the calculation of volume-to-weight factor, for the conversion of amount of food consumed (ml) into weight (g). All food samples were dried in dry air ovens at 70 ^{0}C. After drying, the dried food samples were weighed and the moisture loss determined (Supplementary information 1) for the conversion of the food consumed and Se concentration from being based on dry weight to on fresh weight (FW) based. All food samples were ground into powder using a washable dry mill blender (Philips – HR2106/01) made of steel blades that do not contain Se. The mill was washed and dried after grinding each sample to avoid cross contamination. The Se content of each of the food samples was then analysed, as described in section 2.3 below.

To complete the assessment of population's Se status, hair was also collected from children and women from the same randomly selected households. Hair was sampled at a distance of ~1 cm from the scalp on the occipital area of the head, using titanium nitride-coated scissors to minimize possible release of contaminating elements. Dyed or bleached hair was exempted (Batzevich 1995). Samples were then cleaned with ethanol to remove dirt, followed by air drying. The results were compared to the reference values for human hair Se concentration of 0.77 mg kg^{-1} (Senofonte et al. 2000), which is in line with other reported reference values of 0.7±0.1 mg kg^{-1} (Miekeley et al. 1998), 0.48 to 1.84 mg kg^{-1} (Axelsson et al. 2001), and 0.40 to 2.00 mg kg^{-1} (Austin and Soloway 2012).

To assess the soil Se status of a study location, soil was sampled as well from the same fields/farms belonging to the randomly selected households. A "W" transect was used to sample soil across the field, with four samples collected at each of the four-line of the "W", at a depth of 0-30 cm using soil probes. Root development of maize and legume crops, which are the staple crops in the region, is confined to the soil layer 16 to 22 cm below the surface, above an existing plough pan (Gao et al. 2010). For each field, the soil samples were mixed together to a composite sample, before a subsample was taken. The soil samples were then air dried and sieved through a 2 mm sieve to remove stones and plant debris.

2.3. Chemical analyses

A preliminary step to the analysis consisted of microwave digestion in closed vessels, which was done across for food, hair and soil samples. Each chemical analysis was then conducted in duplicate to check the reproducibility of the method. Each batch of samples was accompanied by external standards, an appropriate standard reference material as shown in Table 2, and a blank to validate accuracy of the Se quantification.

For total soil Se analysis, an amount of 0.25 g soil was placed in Teflon vessels (Omni vent XP1500, CEM, NC, USA) and 6 ml 67-69% Pico-Pure HNO_3 (Chem-Lab, Zedelgem, Belgium) and 2 ml 48% HF (Merck KGaA, Darmstadt, Germany) added. The samples were sonicated for 15 minutes followed by microwave digestion in closed vessels (Mars6, CEM, NC, USA) in a one-step program: Ramp 19 minutes, hold 15 minutes, temperature 200 ^{0}C, pressure 500 PSI, and power 1500 W. After cooling to below 50 ^{0}C, the Teflon vessels were opened carefully and 22 ml 4% H_3BO_3 added to neutralize the HF. The mixture was then microwave digested again in a one-step neutralization program: Ramp 15 minutes, hold 5 minutes, temperature 200 ^{0}C, pressure 500 PSI, and power 1500 W. The method of Zhao and McGrath (Zhao and McGrath 1994) was used for extractable soil Se analysis. A suspension of 30 ml 0.016 M KH_2PO_4 (Merck KGaA, Darmstadt, Germany) and 10 g soil sample was shaken in polycarbonate centrifuge tubes for 1 hour, followed by centrifugation for 20 minutes at 2200 rpm. The clear solution was transferred to autosampler tubes for analysis. A modified Kjeldahl method was used for total soil nitrogen, and an ashing method for percentage organic matter (OM), organic carbon (OC), and $CaCO_3$ analysis. Based on the "Van Bemmelen factor" percentage, soil OC was estimated as OM/1.724 (Soil Survey Staff. 2011). Soil pH in water and KCl were analysed with an A111 pH Benchtop Meter - Thermo Scientific (Carter and Gregorich 2007).

Table 2: Food and soil reference materials analysed in the study and mean extraction recovery observed

Standard reference material		Recovery		
Name	**Code**	**(%)**	**SD**	**Study samples**
Rice flour	NIST1568a	94.9	3.6	Cereals, tubers
Spinach leaves	SRM1570a	103.4	6.6	Leafy vegetables
Fish muscle	ERM-BB422	103.2	3.0	Fish, sea food
Skimmed milk powder	ERM-BD151	99.9	5.2	Milk & milk products
Bovine muscle	ERM-BB184	97.8	5.4	Beef, pork, chicken, egg
Human hair	ERM-DB001	99.1	4.2	Children & women hair
Loamy clay 1	CRM052-50G	98.2	0.8	Soil
Estuarine sediment	BCR-277R	103.6	2.1	Soil
Clean soil	RCT-CLNSOIL3	104.7	1.4	Soil

For food samples, an amount of 0.5 g sample was placed in Teflon vessels and 10 ml 67-69% Pico-Pure HNO_3 added. The samples were sonicated for 15 minutes followed by microwave digestion

in closed vessels using a one-step program: Ramp 25 minutes, hold 15 minutes, temperature 190 ^{0}C, pressure 800 PSI, and power 1200 W. The sample preparation procedure for hair samples was similar to the one used for food samples except for the amount of hair sample being 0.3 g.

After microwave digestion, the clear digests of soil, food, and hair samples were diluted to 50 ml with milli-Q water (Merck KGaA, Darmstadt, Germany) and later analysed with an inductively coupled plasma mass spectrometer (ICP-MS, ELAN DRC-e, Perkin Elmer SCIEX, Waltham, MA, USA), fitted with cyclonic spray chamber with a Babington nebulizer, and CH_4 as the reaction gas. During analysis, samples were introduced from a covered autosampler (AS93plus, Perkin-Elmer) at a rate of 1 ml min^{-1}. Internal standards ^{103}Rh/^{69}Ga (10 µg L^{-1}) were added inline, in 2% C_4H_9OH. The parameters of the ICP-MS system used during Se analysis are shown in Table 3 below. To evaluate the presence of interferences, Se concentration was measured for various isotopes at mass 77, 78, 80, and 82. Final results were based on Se^{80}, the most abundant isotope in nature, and thus suitable for detection of low Se concentration. Limit of quantification for Se was calculated as 10 times the standard deviation of 10 procedure blanks divided by the slope of the standard curve, and was equal to 0.04 µg L^{-1}, and limit of detection as 3.3 times the standard deviation of 10 procedure blanks divided by the slope of the standard curve, and was equal to 0.01 µg L^{-1} (Ich, 2005). Major soil and food elements i.e. Fe, Zn, Al, Mn, Co, Cr, Cu, Ni, Ca, Mg, P, S, Na, and K, were analysed with an inductively coupled plasma optical emission spectrometer (ICP-OES, Vista-MPX, Varian, Palo Alto, CA, USA), using the same digests as used for Se concentration analysis.

Table 3: Optimized ICP-MS system parameters for Se analysis

ICP-MS instrument	ELAN DRC-e, Perkin-Elmer SCIEX
Isotopes monitored	^{77}Se, ^{78}Se, ^{80}Se, ^{82}Se
Power	1250 W
Reaction gas (CH_4) flow rate	0.9 mL min^{-1}
Plasma Ar flow	15 L min^{-1}
Dwell time for each isotope	0.1 s

CHAPTER 3: SELENIUM DEFICIENCY RISK IN CENTRAL KENYA HIGHLANDS – AN ASSESSMENT FROM THE SOIL TO THE BODY

3.1. Introduction

Micronutrient deficiencies form an important global health issue, due to their adverse effects on key development outcomes. They contribute to over 50% morbidity and mortality cases among children, with causal factors ranging from persistent food insecurity to parasites and infectious diseases (MoH et al. 2013). Intervention measures for micronutrients such as Zn and I already exist, with policies on the fortification of processed flour with Zn, and iodization of common table salt (Micronutrient Initiative 2009). However to date, Se deficiency has not been studied nor considered as important from a public health standpoint. In particular, no study has investigated the causes and potential solutions to dietary Se deficiency in Kenya. This is due to two main reasons. First, Kenya is characterized by a lack of representative national dietary intake data and incomplete food composition tables (FCTs). As a result, local nutrition researchers and dietitians are compelled to borrow nutrient values from foreign FCTs, which impedes accuracy of research estimates (Chege and Ndungu 2016). Indeed, FCTs generally vary across countries due to differences in animal or plant species analysed, soil mineral content, agricultural practices, or food processing and preparation methods (Williamson 2005). Second, Se research in Kenya faces analytical limitations which requires specialized equipment and technical support (Pannier et al. 2007; Ralston et al. 2010).

High population in the highlands leads to pressure on land, which diminishes soil fertility and capacity to sustain food production, resulting in persistent food insecurity. The majority of the rural population lives on a poorly diversified diet, based on a few cereal grains from their subsistence farms (Kennedy et al. 2007). A wide variety of soil types are used for agriculture in Kenya, varying in geochemical characteristics and fertility due to differences in geology, relief and climate (Giachene and Kimaru 2003). Selenium occurs naturally in soils, however, its interaction with soil characteristics influences its uptake by plants and concentration in edible crop parts (De Temmerman et al. 2014). Soil Se mobility and availability are controlled by numerous chemical and biochemical processes including sorption, microbial activity, formation of organic and inorganic complexes, precipitation, dissolution, and methylation to volatile compounds (Broadley et al. 2006, Christophersen et al. 2012). After uptake by plants, Se translocation and distribution in edible parts depends upon the plant's accumulation capacity and stage of growth (Zhao et al. 2005). The importance of a relationship between soil characteristics and Se concentration in food is more pronounced for such rural populations, whose diets depend mostly on local cereal grains (Gibson and Hotz 2001).

Using the central Kenya highlands as a case study, the present research focuses on identifying the populations at risk of Se deficiency and on establishing the relationships between the soil geochemical characteristics and Se concentration of local foods, and between dietary Se intake and individuals' Se status. The study first explores Se concentration in various agricultural soil types as well as the main soil factors influencing Se uptake by crops and hence Se concentration in the

foods. The study then provides an understanding of average dietary Se intake of the local population by analysing dietary intake and Se concentration of consumed local foods. Finally, the study assesses hair Se concentrations to evaluate individual Se status and investigates its relationship with Se supply of foods and average dietary Se intake.

3.2. Material and methods

3.2.1. Target area

The Central Kenya Highlands was chosen for the survey because on the one hand, the area is characterized by a high volume of staple food production (International Livestock Research Institute 2015), meaning that reliance of population's diet on local foods is high, increasing the possibility of understanding the link between soils, diet, and target population's Se status. On the other hand, the region is home to a significant number of malnourished children (MoH et al. 2013), which might partly be related to soils - diet nutrient deficiency. Moreover, the area presents a high ethnic diversity, characterized by different cultures, which is reflected in their dietary choices. Thus, the study potentially contributes to the establishment of reference average dietary intakes and corresponding estimated Se intakes across different communities in the region. Furthermore, the area contains a wide range of soil types that differ in geology, relief, and climate (Giachene and Kimaru 2003), which are likely to differ in their Se composition. In order to account for this heterogeneity in soil types and to allow comparative analysis of soil Se status across different soils, eight locations were selected for the study, all characterized by a different agricultural soil type. They included, Mbuyu, Kiaga, Marimanti, Kibirichia, Mbeu, Kiguchwa, Ruiri, and Njoune (Figure 4).

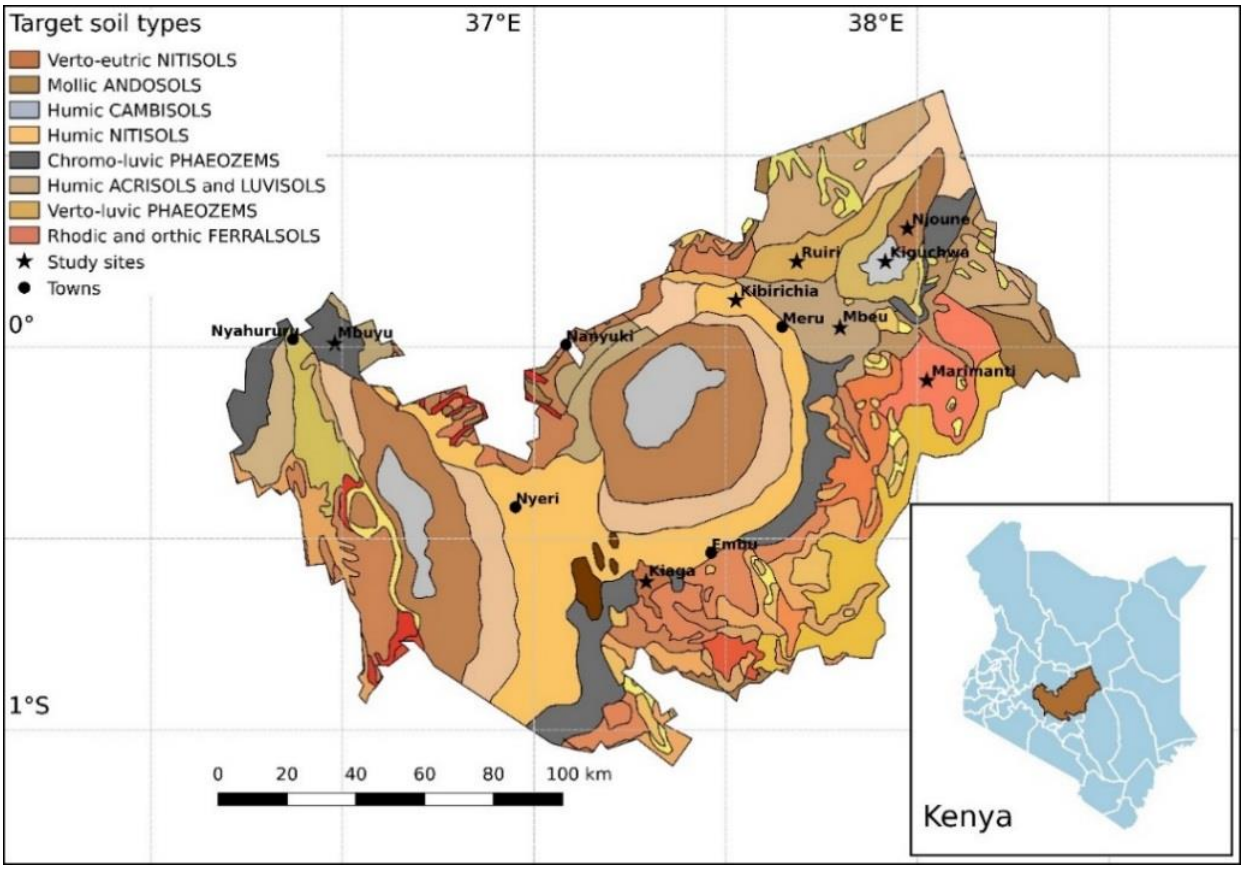

Figure 4: Target agricultural soil types and study locations in Central Kenya Highlands

3.2.2. Target population and sample selection

The study investigated the risk of dietary Se deficiency among 159 children aged 6 to 59 months and 111 women aged 19 to 39 years in rural areas of the study area. In Kibirichia (the first study location to be surveyed), only children were assessed due to limited resources. However, this shortcoming was resolved for the rest of the study locations. Locations within the target agricultural soils were identified based on the regional soil maps (Jaetzold et al. 2006). Sampling of villages in each location was done through simple random sampling. The outcome measure of the survey is the proportion of the study's population that is at a risk of Se deficiency. Sample size (n) for the survey is estimated based on the formula below for proportion estimation with specified absolute precision.

$$n = \frac{Z_{\frac{\alpha}{2}} (1 - P)}{d^2},$$

Where P is the anticipated population proportion, d is absolute precision i.e. total percentage points of error tolerated on each side of the proportion obtained, Z is a constant, its value for a two-sided-test is 1.96 for 95% confidence level, α is the level of significance i.e. the maximum p-value allowed in a problem. The risk of Se deficiency in the East African region has been reported at 52%. An absolute precision of 5% is considered as a reasonable tolerable error. Conventionally, a p-value

of 0.05 and below have been regarded as sufficiently low to be taken as reasonable to indicate a statistically significant difference. Using the formula above, the sample size is estimated to be 428 children or women, after allowing 10% attrition. The sample size was allocated to the eight study locations i.e. 54 households per location. Due to lack of local residents register data, households with under-5 year old children from each village was identified by means of a systematic door-to-door survey and assigned an identification number. Using a random number table, households to be visited were selected for each trial group. All of these (and only these) households will be visited. In case a household declined to participate, it will be replaced by the next household on the list. Individuals with congenital or chronic abnormalities impairing feeding pattern, severely ill, clinical conditions under feeding regimes deviating from a regular diet, or non-permanent residents of the study area were excluded from participating in the study. All subjects were informed about the study objectives, procedures and measurement methods, and signed a consent form. The survey was approved and cleared by Kenyatta National Hospital Ethics & Research Committee (KNH/UoN - ERC) - Nairobi, Kenya (KNH-ERC/A/209), and University Hospital (UZ) Medical Ethics Committee of Ghent University, Belgium (B670201524432).

3.2.3. Assessment of Se status in soil, foods, hair and the dietary intake

The methods related to dietary intake assessment and food, hair, and soil sampling, and the chemical elemental analysis procedures are described in Chapter 2 section 2.3. Data collection and sampling were conducted at the same time for each household. Dietary intake assessment was conducted on women and mothers/guardian on behalf of their children. Soil and foodstuffs were sampled from the subsistence farms or available stock in the same selected households respectively, and hair was sampled from children and women within the target age groups in the same households. The survey was conducted during or after the harvesting season. Most of the food samples were therefore sampled directly from the selected household's farms. Due to postharvest loses, subsistence farmers sell their cereal grains to retail traders in local trading centers, who own storage facilities. The households then re-purchase the same grains overtime, but a higher price. Each trading center serves villages within a radius of 6 to 10 km.

Statistical analysis was performed with SPSS - IBM Corp. (2016). IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY. Normal distribution of data was verified by Shapiro-Wilk Test. Descriptive statistics analysis for Se concentration in food, hair, and soil and dietary intake data were based on untransformed data. Assumptions for linearity and homogeneity of variance were met. To account for the clustered nature of the data, two-level mixed models for clustered data (study location as level 2 variable) were used to determine the relation between Se concentrations in local foodstuffs (Level 1 – dependent variable) and soil characteristics (level 1 predictor variable), and between dietary Se intake (Level 1 – dependent variable) and hair Se concentration (level 1 predictor variable).

3.3. Results

3.3.1. Soil geochemical characteristics

The total soil Se concentration significantly varied across the eight study locations (p-values < 0.001), on average from 0.215 mg kg^{-1} in Marimanti to 0.703 mg kg^{-1} in Njoune (Table 4). The overall mean total soil Se concentration was 0.465 mg kg^{-1}. KH_2PO_4-extractable soil Se concentration ranged from 0.005 mg kg^{-1} in Mbeu to 0.010 mg kg^{-1} in Ruiri, and poorly correlated with the total Se concentration (PCC = 0.389, p-value < 0.001). In reference to the mean total soil Se, only 1.82% of Se was available for plants uptake. The mean concentration of other soil geochemical characteristics also significantly varied across the study locations (p-values < 0.001). Compared to other study locations, Mbuyu and Marimanti had the lowest soil OM, Mn, Fe, Al, and P. In particular, Mbuyu had the lowest soil P concentration of 256 mg kg^{-1}, as compared to 2677 mg kg^{-1} in Njoune and 2653 mg kg^{-1} in Ruiri.

3.3.2. Selenium concentration in foods (mg kg-1) and dietary Se intake (µg d-1)

The most common staple foods consumed across the study locations included maize, beans, potatoes, and green bananas. Rice and wheat products were also important foods in the region. Selenium concentration in foods consumed by the children and women varied significantly across the study locations (p-values < 0.001) as shown in Table 6. Mbuyu reported the lowest Se concentrations in foods: 0.005 mg kg^{-1} in maize, beans, and potato, and 0.001 mg kg^{-1} in green banana (on fresh weight basis). Ruiri on the other hand reported the highest Se concentration in foods: 0.047 mg kg^{-1} in maize, 0.030 mg kg^{-1} in beans, 0.028 mg kg^{-1} in potato, and 0.012 mg kg^{-1} in green banana. Generally, consumption of good Se sources such as animal-source foods such as fish, meat, and eggs was limited. Milk was the only animal-source food that were routinely consumed across the study locations, however, it had low Se concentration ranging from 0.009 to 0.043 mg kg^{-1}.

Variation in geochemical characteristics between study locations was not significant (p-values > 0.05). The geochemical characteristics that significantly explained Se concentration in foods (p-values < 0.001) are shown in Table 5. This included soil P and Se for maize, beans, and amaranthus leaves; soil pH and Se for beans, potatoes, and amaranthus leaves; soil OM and Se for beans and potatoes; soil Fe and Se for potatoes; KH_2PO_4-extractable/Ca and Se for potatoes and amaranthus leaves. Generally, an increase in these soil characteristics was likely to result into an increase in Se concentration in the respective foods, except for soil Fe and Ca which were likely to result in a decrease of Se concentration. Notably, no significant relationship was observed between soil Se status and Se concentration in foods except between KH_2PO_4-extractable soil Se and the Se concentration in amaranths leaves.

Table 4: Soil geochemical characteristics (mean with standard deviation between brackets) at the 8 study locations in Kenya

Parameter	Marimanti (n =24)	Mbuyu (n = 22)	Mbeu (n = 26)	Kiaga (n = 17)	Kibirichia (n = 25)	Kiguchwa (n = 13)	Njoune (n = 20)	Ruiri (n = 32)
Total Se (mg kg^{-1})	0.215	0.466	0.372	0.392	0.555	0.602	0.703	0.417
	(0.082)	*(0.148)*	*(0.067)*	*(0.094)*	*(0.167)*	*(0.179)*	*(0.159)*	*(0.094)*
Extractable Se	0.008	0.009	0.005	0.007	0.009	0.008	0.009	0.010
(mg kg^{-1})	*(0.002)*	*(0.001)*	*(0.001)*	*(0.001)*	*(0.003)*	*(0.002)*	*(0.001)*	*(0.003)*
Organic matter	0.7	1.2	1.7	1.6	1.9	2.2	2.3	1.8
(% OM)	*(0.1)*	*(0.1)*	*(0.1)*	*(0.1)*	*(0.2)*	*(0.2)*	*(0.2)*	*(0.2)*
CaCO3 (%)	0.2	0.4	0.7	0.5	1.0	0.7	0.6	0.6
	(0.1)	*(0)*	*(0.2)*	*(0.1)*	*(0.1)*	*(0.1)*	*(0.1)*	*(0.1)*
pH-H_2O	6.9	6.3	5.8	6.1	6.3	5.9	6.5	6.6
	(0.2)	*(0.4)*	*(0.3)*	*(0.3)*	*(0.3)*	*(0.4)*	*(0.2)*	*(0.3)*
pH-KCl	6.2	4.7	4.7	4.9	5.1	4.7	5.2	5.4
	(0.4)	*(0.5)*	*(0.3)*	*(0.4)*	*(0.4)*	*(0.3)*	*(0.3)*	*(0.4)*
Mn (g kg^{-1})	0.8	1.8	4.5	3.1	3.1	3.5	3.8	2.1
	(0.6)	*(0.9)*	*(0.6)*	*(1.2)*	*(0.7)*	*(1.0)*	*(0.6)*	*(0.5)*
Fe (g kg^{-1})	58	56	116	114	100	91	88	103
	(34)	*(12)*	*(22)*	*(20)*	*(14)*	*(20)*	*(22)*	*(9)*
Al (g kg^{-1})	42	43	88	101	87	106	109	97
	(23)	*(11)*	*(9)*	*(12)*	*(8)*	*(10)*	*(9)*	*(10)*
Zn (mg kg^{-1})	51	91	161	129	114	144	199	92
	(35)	*(22)*	*(45)*	*(28)*	*(29)*	*(29)*	*(45)*	*(22)*
Co (mg kg^{-1})	23	12	72	41	42	48	44	44
	(16)	*(2)*	*(21)*	*(11)*	*(11)*	*(21)*	*(21)*	*(5)*
Cr (mg kg^{-1})	82	27	128	43	109	97	75	104
	(75)	*(7)*	*(45)*	*(25)*	*(36)*	*(37)*	*(45)*	*(15)*
Cu (mg kg^{-1})	36	11	69	33	43	51	55	50
	(27)	*(2)*	*(25)*	*(9)*	*(11)*	*(25)*	*(25)*	*(6)*
Ni (mg kg^{-1})	42	16	107	33	105	93	81	85
	(22)	*(3)*	*(43)*	*(18)*	*(38)*	*(41)*	*(43)*	*(11)*
S (mg kg^{-1})	119	225	256	292	436	355	479	270
	(55)	*(51)*	*(115)*	*(122)*	*(90)*	*(37)*	*(115)*	*(66)*
Na (mg kg^{-1})	161	113	77	164	182	126	171	123
	(58)	*(21)*	*(35)*	*(112)*	*(35)*	*(65)*	*(35)*	*(31)*
K (mg kg^{-1})	2439	2771	1014	1057	2840	1850	2310	2119
	(654)	*(518)*	*(562)*	*428()*	*(890)*	*(669)*	*(35)*	*(478)*
Ca (mg kg^{-1})	2785	2250	1904	2737	3851	1444	2761	3068
	(1289)	*(1437)*	*(1085)*	*(1091)*	*(1573)*	*(698)*	*(1085)*	*(1137)*
Mg (mg kg^{-1})	3025	1483	2497	2610	4445	2402	3093	2361
	(905)	*(292)*	*(1206)*	*(699)*	*(1235)*	*(1343)*	*(1206)*	*(1110)*
P (mg kg^{-1})	660	256	2072	2711	2280	1588	2677	2653
	(445)	*(98)*	*(886)*	*(1411)*	*(894)*	*(506)*	*(886)*	*(814)*
N (mg kg^{-1})	1436	2135	1905	2335	3886	2460	3367	2029
	(598)	*(470)*	*(970)*	*(1088)*	*(593)*	*(478)*	*(970)*	*(560)*

Table 5: Significant linear regression equation coefficients (β) between soil characteristics and Se concentration in food regression (p-value < 0.05)

Se Conc.	n	pH	P	OM	Fe	[1]Ex-Se	Ca
Bean	97	1.68	0.049	0.007	-	-	-
Potato	49	2.25	-	0.011	-0.22	-	-
Maize	130	-	0.072	-	-	-	-
Amaranthus	48	0.22	0.137	-	-	7.34	-0.42

n: sample size. [1]Ex-Se: Soil extractable Se concentration

3.3.3. Population characteristics and estimated average dietary Se intake for children and women

The average age of the study population was 2.5 years for children (SD = 1.3) and 28.9 years for women (SD = 7.4). Among the children, 55% were girls and 45% boys. In total, the study found that 41% of children were stunted, 15% underweight, and 2% wasted, based on weight and height measurements. Stunting was therefore observed to be of greater magnitude than underweight and wasting. For women, overweight was observed to exceed underweight based on the body mass index, with 28% of women being overweight and 12% being underweight. Majority of the women (63%) received only the primary education, 30 % received secondary education, and 7 % received tertiary education. Most (90%) of the women were subsistence farmers or housewives, with the remaining 10% being employed or self-employed.

The dietary Se intake from the foods consumed was based on the Se concentration (mg kg^{-1}) in Table 6 and the amount consumed (g) in Table 7 for children and Table 9 for women. Generally, the main dietary Se sources for both children and women across the study locations were bean, bread, maize, milk, potato, and rice as shown in Table 8 for children and Table 10 for women. The average dietary Se intake from all foods consumed varied significantly across locations from 7.6 µg day^{-1} in Mbuyu to 23.4 µg day^{-1} in Kibirichia among the children and from 14.4 in Mbuyu µg day^{-1} to 52.6 µg day^{-1} in Kiguchwa among women (p-values < 0.001). Intuitively and in line with Se concentration in foodstuffs, Mbuyu reported the lowest average dietary Se intake, followed by Marimanti at 14.7 µg day^{-1} for children and 30.1 µg day^{-1} for women (Table 11). All the children and women assessed in the two locations had inadequate dietary Se intake based on EARs. Among the children, study locations including Kibilichia, Kiguchua, Njoune, and Ruiri, which reported high Se concentration in staple foods, had adequate dietary Se intake. Mbeu and Kiaga reported sub-optimal dietary Se intake. However for women, all the study locations reported inadequate average dietary Se intake except in Kiguchwa. Overall, the dietary Se intake of 87% children and 97% women was below EARs.

3.3.4. Selenium concentration in the hair

Selenium concentration in the hair varied across the study locations from 0.285 mg kg^{-1} in Mbuyu to 0.632 mg kg^{-1} in Ruiri among children, and from 0.243 mg kg^{-1} in Mbuyu to 0.665 mg kg^{-1} in Kiguchwa among women. In line with the average dietary Se intake, Mbuyu, Marimanti, Kiaga, and Mbeu had lower Se concentrations in hair for both children and women, as compared to study locations with higher hair Se concentration such as Kigucwha, Njoune, and Ruiri. In total, average hair Se concentration was 0.536 mg kg^{-1} among children and 0.546 mg kg^{-1} among women (Table 12). As a consequence, 92% of the children and 94% of the women had hair Se concentration below the reference value. The ranking of locations based on Se concentration in hair was therefore similar to the one for dietary Se intake. Notably, the mean hair Se concentration among girls was

0.546 mg kg^{-1} and 0.540 mg kg^{-1}among boys, respectively, with no significant differences between girls and boys (T-test comparison of mean: p-value = 0.94). The study found that the between cluster variation in hair Se concentration was not significant (p-values > 0.05). Dietary Se intake from maize was positively associated with Se concentration in hair for women (p-value = 0.002). Generally, an increase in dietary Se supply from maize grains was likely to result into an increase in Se concentration in hair for women.

Table 6: Se concentration (mean and standard deviation (mg kg^{-1} FW) in different foodstuffs consumed by children and women at the 8 study locations in Kenya

	Mbuyu			Marimanti			Mbeu			Kiaga			Kibirichia			Kiguchwa			Njoune			Ruiri		
	Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
Amaranthus				0.021		1	0.045	0.030	7	0.048	0.034	4	0.059		1	0.029		1	0.003		1	0.157	0.103	8
Banana	0.001		3	0.016	0.009	5	0.008	0.008	14	0.018	0.012	4	0.029	0.001	13	0.016	0.016	5	0.025	0.012	9	0.012	0.008	14
Bean	0.005	0.003	12	0.006	0.002	6	0.014	0.007	18	0.023	0.018	9	0.018	0.010	11	0.013	0.007	5	0.020	0.016	12	0.030	0.016	18
Bread	0.013	0.002	11	0.012	0.003	3	0.017	0.003	6	0.012	0.002	6	0.017	0.001	15	0.019		1	0.016	0.003	7	0.016	0.003	18
Cabbage	0.030		5	0.030		1	0.030		1	0.030		1	0.030		1				0.030		1	0.030		1
Cooking fat	0.004		1	0.004		1	0.004		1	0.004		1	0.004		1	0.004		1	0.004		1	0.004		1
Cooking oil	0.005		1	0.005		1	0.005		1	0.005		1	0.005		1	0.005		1	0.005		1	0.005		1
Cowpea leaves				0.026		1	0.038	0.014	9				0.020	0.022	5	0.033	0.024	6	0.067	0.004	2	0.046		1
Kales	0.049	0.049	9				0.037		1	0.087	0.003	9	0.035	0.020	4							0.037		1
Maize (grain)	0.005	0.003	8	0.029	0.019	9	0.024	0.015	24	0.024	0.013	8	0.020	0.020	18	0.051	0.021	5	0.039	0.017	9	0.047	0.027	15
Maize (Ugali)	0.004	0.003	12	0.024	0.014	2	0.020	0.012	11	0.015	0.007	10	0.017	0.020	9	0.038		1	0.031	0.018	4	0.039	0.022	12
Milk	0.009		1	0.041	0.008	14	0.013		1	0.018		14	0.013		1	0.010		1	0.015		1	0.041		1
Millet	0.043		1	0.033	0.013	10	0.029	0.013	18	0.023	0.016	7	0.029	0.010	8	0.030	0.018	2	0.029	0.011	3	0.027	0.015	12
Onion	0.008	0.000	18	0.008	0.000	12	0.018	0.007	21	0.008	0.000	13	0.014	0.001	17	0.025		1	0.023		1	0.013	0.005	26
Pigeon pea				0.016		1	0.022	0.009	4							0.019		1				0.027	0.017	5
Potato	0.005	0.002	16	0.014	0.021	5	0.008	0.006	11	0.006		2	0.025	0.020	16	0.015	0.005	7	0.036	0.014	11	0.028	0.010	16
Rice	0.012	0.003	8	0.012	0.003	6	0.013	0.005	11	0.012	0.007	10	0.014	0.001	11	0.017	0.006	4	0.014	0.005	7	0.012	0.002	19
Salt	0.015		1	0.015		1	0.015		1	0.015		14	0.015		1	0.015		1	0.015		1	0.015		1
Sorghum				0.054	0.026	8	0.042	0.022	3	0.011	0.005	6	0.042	0.020	4				0.055	0.076	3	0.027		1
Sugar	0.011		1	0.011		1	0.011		1	0.011		14	0.011		1	0.011		1	0.011		1	0.011		1
Tea leaves	0.157		1	0.157		1	0.157		1	0.157		14	0.157		1	0.157		1	0.157		1	0.157		1
Tomato	0.001		1	0.003	0.002	5	0.004		1	0.009	0.006	9	0.004		1	0.004		1	0.007		1	0.007		1

FW: Fresh weight basis. SD: Standard deviation. n: number of food samples. Ugali: most common maize meal – thick porridge

Table 7: Amounts of different foodstuffs taken in by children (mean and standard deviation, g FW day^{-1}) at the 8 study locations in Kenya

	Mbuyu			Marimanti			Mbeu			Kiaga			Kibirichia			Kiguchwa			Njoune			Ruiri		
	Mean	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
Amaranthus				12.2		1	18.5	8.46	7	19.2	0.60	4	7.56		1	24.4		1	6.10		1	10.7	10.6	8
Banana	51.8	43.6	3	76.2	21.1	4	81.3	53.1	13	101	36.5	4	51.8	24.3	14	86.9	62.1	5	76.4	33.5	10	79.6	46.6	14
Bean	112	78.9	12	150	80.9	5	129	104	17	81.8	30.5	9	67.0	47.4	12	184	121	5	110	87.1	13	87.9	72.3	18
Bread	73.2	41.6	11	94.8	31.8	3	49.4	17.3	5	85.0	54.1	6	135	94.3	16	91.2		1	112	87.4	7	132	116	18
Cabbage	56.4	32.0	5	99.8		1	65.4	53.5	2	55.0	14.0	4	68.8	48.6	2				67.1	40.4	4	82.1	60.3	8
Cooking fat	8.23	5.11	16	5.91	5.03	8	3.75	3.00	16	4.77	3.15	3	5.10	5.66	7	3.56	1.00	5	4.70	6.00	5	3.36	3.23	15
Cooking oil				13.9	10.2	3	4.79	3.22	4	14.7	8.26	12	11.0	10.5	12				3.47	2.32	6	5.47	4.40	13
Cowpea leaves				17.5		1	29.0	18.6	9				14.6		1	34.8	24.4	6	11.0	11.4	2	43.8	20.6	2
Kales	16.9	10.7	8				33.8	27.6	4	28.4	9.7	9	27.7	25.9	4							28.2		1
Maize (grain)	67.4	74.7	5	81.6	85.6	8	101	91.4	23	44.9	26.4	7	68.2	44.8	18	139	136	5	126	120	10	23.0	24.6	14
Maize (Ugali)	111	71.3	12	86.2	66.5	2	127	102	11	120	25.6	10	183	96.5	9	235		1	156	168	4	167	138	12
Milk	164	118	20	106	75.1	9	135	118	24	156	90.1	12	384	182	24	210	132	7	151	149	13	222	194	32
Millet	2.28		1	28.4	27.5	10	42.8	32.2	17	20.2	18.4	7	55.4	33.0	9	24.8	3.80	2	40.5	30.6	3	15.5	12.0	12
Onion	3.86	4.44	18	2.11	1.15	12	4.92	5.15	20	6.09	3.94	13	5.40	4.23	18	6.95	7.03	5	3.41	4.82	10	4.72	3.19	26
Pigeon pea				200		1	60.0	32.7	4							4.00		1				105	82.4	5
Potato	86.0	52.1	16	40.3	17.2	4	73.7	57.7	10	90.3	26.4	2	92.1	48.9	17	117	69.2	7	77.1	73.8	12	74.7	79.9	16
Rice	121	64.0	8	106	58.6	5	141	132	11	116	25.5	10	127	82.9	11	162	121	4	198	109	8	143	88.5	19
Salt	1.90	1.6	11	1.90	2.07	13	2.6	2.00	22	4.08	2.50	12	3.23	2.18	24	1.33	0.92	9	2.34	1.42	12	3.18	2.17	30
Sorghum				12.7	12.6	8	36.9	23.7	3	39.7	39.2	6	75.8	38.1	4				42.6	10.5	3	6.35	3.75	3
Sugar	20.9	16.6	20	13.1	9.32	11	23.5	21.1	20	23.7	16.3	13	22.5	17.6	19	37.9	29.1	6	9.33	6.15	13	20.8	17.8	27
Tea leaves	0.62	0.44	20	0.65	0.46	9	1.10	0.66	18	0.57	0.50	11	1.12	0.51	13	1.41	0.65	6	0.67	0.43	13	0.99	0.54	29
Tomato	20.1	17.1	12	24.0	19.3	4	20.7	8.20	7	25.6	22.0	9	20.2	30.3	5	1.96		1	33.8	18.6	9	17.0	15.3	18

FW: Fresh weight basis. SD: Standard deviation. n: number of food samples. Ugali: most common maize meal – thick porridge

Table 8: Daily Se intake by children through individual foodstuffs consumed (mean and standard deviation, µg day^{-1}) at the 8 study locations in Kenya

	Mbuyu			Marimanti			Mbeu			Kiaga			Kibirichia			Kiguchwa			Njoune			Ruiri		
Foodstuff	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
Amaranthus				0.251		1	0.915	0.919	7	0.906	0.607	4	0.449		1	0.715		1	0.020		1	1.123	1.090	7
Banana	0.060	0.051	3	1.136	0.947	4	0.471	0.395	13	1.834	1.325	4	1.473	0.690	14	0.803	0.448	5	1.511	0.663	9	0.929	0.909	14
Bean	0.542	0.404	12	0.888	0.561	5	1.488	1.000	17	2.029	1.688	9	1.150	1.063	12	1.277	0.583	4	1.147	0.562	13	1.959	1.373	17
Bread	1.067	0.731	11	1.462	0.413	3	0.843	0.314	5	0.943	0.408	7	2.615	1.735	16	1.712		1	1.616	1.009	7	2.049	1.395	18
Cabbage	0.985	0.558	5	0.358		1	0.102	0.084	2	0.131	0.082	4	1.906	1.637	2				0.241	0.145	4	0.295	0.217	8
Cooking fat	0.028	0.016	15	0.022	0.019	8	0.014	0.011	16	0.018	0.012	3	0.019	0.021	7	0.013	0.004	5	0.017	0.022	5	0.012	0.012	15
Cooking oil				0.067	0.049	3	0.023	0.015	4	0.070	0.040	12	0.052	0.050	12				0.017	0.011	6	0.026	0.021	13
Cowpea leaves				0.454		1	1.078	0.716	9				0.314		1	0.909	0.503	6	0.707	0.714	2	2.006	0.946	2
Finger millet				0.142		1	0.333	0.256	12	0.174	0.166	3	1.010	0.394	4	0.632	0.675	3	0.592		1	0.199	0.141	6
Kales	0.628	0.825	8				1.262	1.031	4	2.475	0.840	9	1.118	1.474	4							1.052		1
Maize	0.434	0.317	13	2.020	1.697	8	2.874	2.543	23	2.093	1.315	12	1.855	2.062	22	3.825	3.564	4	4.023	4.679	10	3.314	3.076	19
Milk (FW)	1.419	1.023	20	4.588	3.255	9	1.721	1.510	24	2.844	1.643	12	4.811	2.279	24	2.150	1.351	7	2.279	2.237	13	5.868	3.791	26
Millet	0.098		1	0.756	0.539	10	1.071	0.921	17	0.238	0.160	7	1.376	0.690	9	0.722	0.321	2	0.943	0.431	3	0.493	0.623	12
Onion	0.030	0.035	18	0.016	0.009	12	0.052	0.052	16	0.046	0.029	13	0.073	0.060	18	0.052	0.012	3	0.047	0.050	9	0.053	0.035	25
Potato	0.412	0.303	16	0.205	0.125	4	0.515	0.468	10	0.543	0.159	2	1.204	1.090	15	1.833	1.170	7	1.676	1.307	10	0.810	0.686	12
Rice	1.539	1.294	8	1.279	0.718	5	1.784	1.538	11	1.299	0.574	10	1.664	0.974	11	2.432	2.141	4	2.685	1.830	8	1.736	1.180	19
Salt	0.029	0.024	11	0.029	0.032	13	0.039	0.031	22	0.062	0.038	12	0.049	0.033	24	0.020	0.014	9	0.036	0.022	12	0.049	0.033	30
Sorghum				0.744	0.865	8	0.782	0.583	2	0.386	0.363	6	1.802	0.728	2				0.529	0.150	2	0.172	0.102	3
Sugar	0.220	0.175	20	0.138	0.098	11	0.248	0.223	20	0.251	0.172	13	0.237	0.186	19	0.400	0.308	6	0.099	0.065	13	0.220	0.188	27
Tea leaves	0.097	0.068	20	0.102	0.072	9	0.172	0.104	18	0.090	0.078	11	0.175	0.079	13	0.220	0.102	6	0.105	0.067	13	0.155	0.084	29
Tomato	0.028	0.024	12	0.033	0.027	4	0.073	0.029	7	0.131	0.121	8	0.071	0.107	5	0.007		1	0.235	0.129	9	0.118	0.106	18

DW: Dry weight basis. FW: Fresh weight basis. SD: Standard deviation. n: number of participants who consumed the foodstuff

Table 9: Amounts of different foodstuffs taken in by women (mean and standard deviation, g FW day^{-1}) at the 7 study locations in Kenya

	Mbuyu			Marimanti			Mbeu			Kiaga			Kiguchwa			Njoune			Ruiri		
Foodstuff	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
Amaranthus							41.6	27.0	3	28.1	19.1	5							27.5	12.9	2
Banana				180	44.5	3	122	93.1	7	123	82.5	4	143.7	126.9	6	169	122	3	126	170	2
Bean	235	198	12	306	210	6	277	136	17	292	128	12	250	182	11	300	146	11	147	88.3	14
Bread	238	124	6	221	147	4	179	82.5	2	219	96.9	7	275	120	3	179	59.3	3	223	109	11
Cabbage	103	68.8	3	86.0		1	158	34.8	3	92.9	58.0	6	138		1	241		1	34.4		1
Cooking fat	12.7	10.7	14	14.6	8.6	10	7.48	5.93	14	34.3		1	8.03	5.37	9	5.56	5.45	6	9.65	8.17	12
Cooking oil				17.8	16.6	4	9.73		1	23.7	17.2	13	6.44		1	10.7	5.31	6	3.92	2.54	6
Cowpea leaves				46.2	18.4	3	44.7	30.9	8				53.5	48.8	8	14.6		1	18.3	5.16	2
Finger millet				16.6		1	11.3	5.58	5	8.59	11.1	2	6.55		1				5.26		1
Kales	52.4	23.8	8				79.9	19.9	2	79.9	35.2	8				89.3	73.1	2	50.1	38.0	3
Maize (grains)	113	86.3	7	252	123	10	237	162	17	140	95.5	7	334	193	10	227	148	10	144	127	10
Maize (Ugali)	230	101	11	235	32.0	4	242	102	11	240	82.3	8	343	158	4	480	97.0	2	184	80.7	10
Milk (FW)	192	136	17	192	198	11	214	203	16	195	125	13	339	168	11	378	212	12	278	212	18
Millet	0.26		1	60.8	59.2	6	48.7	51.4	5	9.21	9.66	4	31.2	21.1	3	50.4	19.6	4	24.3	34.7	4
Onion	7.35	6.98	16	5.8	2.70	14	6.55	4.98	15	12.0	6.70	14	7.88	5.61	10	4.80	3.80	12	11.4	7.00	19
Pigeon pea				440		4	440		4				440		1				440		7
Potato	163	96.6	13	52.6	18.0	3	128	82.5	6	81.0	29.50	5	95.0	35.9	10	179	115	10	102	112	11
Rice	160	162	7	210	74.2	5	303	113	7	313	139	10	245	99.0	2	276	130	8	181	80.0	13
Salt	3.35	2.92	10	4.58	3.30	12	5.32	3.31	12	5.55	4.32	11	6.18	5.41	10	4.10	3.12	12	4.18	2.72	16
Sorghum				24.2	13.6	6	31.6		1	24.6	26.7	3							11.1	0.83	2
Sugar	37.0	21.9	13	24.5	12.6	13	33.8	23.4	15	23.2	12.0	13	39.7	28.5	9	29.1	29.0	12	29.4	21.4	15
Tea leaves	1.31	0.72	15	0.87	0.30	13	1.37	0.66	13	0.86	0.47	13	1.88	0.93	11	1.07	0.62	10	1.43	0.92	18
Tomato	33.4	29.4	10	39.9	22.1	5	64.8	28.3	5	42.8	27.8	7	51.2	25.9	4	55.0	34.5	10	34.6	16.5	11

FW: Fresh weight basis. SD: Standard deviation. n: number of food samples. Ugali: most common maize meal – thick porridge. Note: women were not assessed in Kibirichia (the first study location to be surveyed) due to limited resources.

Table 10: Daily Se intake by women through individual foodstuffs consumed (mean and standard deviation, µg day^{-1}) at the 7 study locations in Kenya

	Mbuyu			Marimanti			Mbeu			Kiaga			Kiguchwa			Njoune			Ruiri		
Foodstuff	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
Amaranthus							1.918	1.637	3	1.341	1.063	5	6.956		1				3.512	2.345	2
Banana				2.653	1.525	3	1.532	2.013	7	1.761	0.304	4	1.951	1.776	6	4.409	3.549	3	1.042	1.349	2
Bean	1.095	1.016	12	1.600	1.181	6	4.194	2.579	17	5.002	4.221	10	2.939	1.879	11	7.152	4.440	11	3.418	1.845	14
Bread	2.831	1.838	6	2.505	1.549	4	2.929	2.153	2	2.151	0.924	7	4.590	2.055	3	2.936	1.313	3	3.382	1.977	12
Cabbage	1.801	1.201	3	0.309		1	1.158	1.177	3	0.996	0.872	6	1.906		1	0.865		1	0.588		1
Cooking fat	0.047	0.039	14	0.054	0.032	10	0.028	0.022	14	0.126		1	0.030	0.020	9	0.020	0.020	6	0.033	0.030	13
Cooking oil				0.085	0.079	4	0.047		1	0.113	0.082	13	0.031		1	0.051	0.025	6	0.019	0.012	6
Cowpea leaves				1.187	0.458	3	1.557	1.251	8				1.083	0.939	8	1.012		1	1.235	0.800	2
Finger millet				0.708		1	0.334	0.274	5	0.102	0.098	2	0.106		1				0.055		1
Kales	2.599	3.289	8				2.980	0.744	2	7.001	3.112	8				2.750		1	1.870	1.417	3
Maize	1.613	1.414	13	6.948	4.501	10	6.315	4.386	20	5.759	7.702	11	17.100	6.316	11	10.384	7.717	11	8.800	7.143	17
Milk (FW)	1.665	1.179	17	5.973	3.863	10	2.728	2.593	16	3.548	2.282	13	3.467	1.718	11	5.694	3.183	12	6.382	4.337	12
Millet	0.010		1	1.451	0.960	5	1.114	0.384	5	0.204	0.110	4	1.281	1.225	3	1.046	0.278	4	0.757	1.090	4
Onion	0.057	0.056	16	0.045	0.021	14	0.080	0.068	13	0.092	0.050	14	0.117	0.061	7	0.089	0.041	11	0.111	0.067	16
Pigeon pea				2.794	2.285	4	2.329	0.579	3				3.743		1				2.666	1.202	7
Potato	0.874	0.538	12	0.253	0.042	3	1.624	1.416	6	0.399	0.235	5	1.263	0.802	10	3.754	1.896	7	1.346	0.915	9
Rice	1.109	0.447	6	2.707	0.948	5	3.973	1.626	7	3.240	2.247	10	5.138	3.878	2	3.002	2.118	7	2.397	1.450	13
Salt	0.051	0.045	10	0.070	0.050	12	0.081	0.051	12	0.085	0.066	11	0.095	0.083	10	0.063	0.048	12	0.064	0.042	16
Sorghum				0.969	0.432	6	2.075		1	0.294	0.242	3							0.301	0.022	2
Sugar	0.391	0.231	13	0.259	0.133	13	0.357	0.247	15	0.246	0.126	13	0.419	0.302	9	0.308	0.307	12	0.311	0.227	15
Tea leaves	0.205	0.113	15	0.136	0.047	13	0.215	0.104	13	0.135	0.074	13	0.294	0.146	11	0.167	0.097	10	0.224	0.144	18
Tomato	0.046	0.041	10	0.055	0.031	5	0.229	0.100	5	0.286	0.090	5	0.181	0.091	4	0.382	0.240	10	0.240	0.115	11

DW: Dry weight basis. FW: Fresh weight basis. SD: Standard deviation. n: number of participants who consumed the foodstuff. Note: women were not assessed in Kibirichia (the first study location to be surveyed) due to limited resources.

Table 11: Dietary Se intake (mean, median and standard deviation, µg day^{-1}) and evaluation against age-specific EARs[a] on the 8 studied locations in Kenya

	Children					Women				
		Se intake (µg day^{-1})			< EARs		Se intake (µg day^{-1})			< EARs
Location	**n**	**Mean**	**SD**	**Median**	**(%)[b]**	**n**	**Mean**	**SD**	**Median**	**(%)[b]**
Mbuyu	20	7.6	0.86	6.3	100	17	14.4	1.32	11.2	100
Marimanti	14	14.7	0.81	1.8	100	14	30.1	1.42	27.7	100
Mbeu	26	15.9	0.80	12.2	92	10	37.5	1.22	31.7	100
Kiaga	14	16.6	0.49	16.2	93	14	32.8	1.01	27.4	93
Kibirichia	27	23.4	0.78	19.9	74	[c]-	-	-	-	-
Kiguchwa	10	17.7	0.59	16.1	90	13	52.6	1.33	51.4	100
Njoune	16	18.5	0.61	15.2	75	13	44.1	1.68	45.4	85
Ruiri	32	22.6	0.39	19.7	72	20	38.7	0.82	32.9	100
Average		17.1	0.67	14.8	87		36.1	1.20	32.6	97

n: sample size. SD: Standard deviation [a]: EARs for children 1 to 3 y = 17 µg d^{-1}, 4 to 8 y = 23 µg d^{-1}; for women = 45 µg d^{-1}. [b]: Percentage of study population with average daily Se intake below EARs. DW = Dry weight basis. [c]: women not assessed

Table 12: Hair Se concentration (mean, median and standard deviation, mg kg^{-1}) for children and women on the 8 studied locations in Kenya

	Children				Women			
Location	**Mean**	**SD**	**Median**	**n**	**Mean**	**SD**	**Median**	**n**
Mbuyu	0.285	0.058	0.282	15	0.243	0.046	0.228	8
Marimanti	0.504	0.058	0.480	7	0.497	0.050	0.505	7
Mbeu	0.554	0.072	0.538	20	0.594	0.089	0.571	16
Kiaga	0.504	0.077	0.488	4	0.502	0.113	0.471	3
Kibirichia	0.574	0.145	0.527	21	[a]-	-	-	-
Kiguchwa	0.606	0.089	0.619	8	0.665	0.094	0.657	9
Njoune	0.627	0.108	0.613	12	0.583	0.086	0.574	5
Ruiri	0.632	0.179	0.589	20	0.642	0.163	0.659	11

n: sample size, SD: Standard deviation

3.4. Discussion

This study assessed the risk of dietary Se deficiency among the rural population in the central Kenya highlands, based on average daily dietary Se intake and individuals' Se status. In order to assess the Se status in different physiological conditions, eight study locations were selected to represent different agricultural soils and geochemical characteristics influencing crop's Se uptake. In this study, Se concentration in foods varied across the study locations but also within the locations. For instance, the Se concentration in maize ranged from 0.001 to 0.010 mg kg^{-1} in Mbuyu. This level of Se content in cereal products is classified as low (Ge and Yang 1993). In other study locations, higher mean Se concentrations were observed in maize, ranging from 0.029 mg kg^{-1} in Marimanti to 0.047 mg kg^{-1} in Ruiri. In this study, there was no significant relationship between the soil Se and Se in foods. Notably, the Se concentration in maize was higher than was reported in Malawi (Chilimba et al 2011). This is mainly explained by the difference in soil Se status between the two countries, with Malawi having much lower total soil Se of 0.1941 mg kg^{-1} and extractable soil Se of 0.0056 mg kg^{-1}, compared to 0.465 mg kg^{-1} and 0.008 mg kg^{-1}, respectively in Kenya.

Although there was no obvious link between grain and soil Se concentration in both studies, most plants (non-accumulators) accumulate Se in direct relationship to the amount available in the soil (Sors et al, 2005). In this study, as well as in Malawi, geochemical characteristics, and especially the soil pH were significantly associated with Se concentration in foods. The mean soil pH in Kenya (6.3) was relatively higher than in Malawi (5 - 6) which further explains the higher Se concentration in maize in Kenya. The mean total soil Se found by Chilimba et al. (2011) in Malawi and in the present study in Kenya are both within the range considered Se deficient of 0.1 to 0.6 mg kg^{-1} (Fordyce 2013). However as demonstrated by Lyons et al. (2004), two soil types with exactly the same total soil Se concentration of 0.08 mg kg^{-1} but different soil pH of 6.6 and 8.6, resulted in 11 folds difference in Se concentration in wheat grains. This evidences the role of soil geochemical interactions in determining Se availability for plants' uptake. In this regard, Christophersen et al. (2012) called for a revision of the total soil Se concentration level that should be considered as Se deficient.

In this study, Se concentration in foods was associated with the soil pH, OM, P and Fe. An increase in soil pH was associated with an increase in Se concentration in beans, potatoes, and amaranths leaves. Across the study locations, the soil pH ranged from moderately acidic (5.8) to neutral (6.9). In nature, Se species present in a soil environment are determined to a large extent by the soil pH and redox potential (Eh) (Mayland et al. 1991). Under most natural redox conditions, selenite (Se^{4+}) and selenate (Se^{6+}) are the predominant inorganic species. This occurs in the oxic zone (0.4-0.8V) as illustrated in the Pourbaix diagram (Figure 1). Within this Eh zone, changes of pH induce transformations between selenite and selenate. At higher redox potential and pH, Se oxidation state shifts to Se^{6+}. The selenate formed is less adsorbed by soil minerals and is therefore available in soil solution for uptake by crops. However at lower pH, the oxidation state shifts to Se^{4+}. The selenite formed is adsorbed by ligand exchange onto soil clay surfaces with greater affinity than selenate, limiting Se concentration in the soil solution (Blaylock and James 1994). The binding strength increases as the pH decreases (Parkman and Hultberg 2002). In addition, selenide and elemental Se that are generally unavailable in the soil solution may occur in the more reducing environments (Christophersen et al. 2012), whereas elemental Se can also occur under less reducing conditions at low pH levels.

The other soil characteristic that explained Se concentration in crops was OM. An increase in soil OM was associated with an increase in Se concentration in beans and potatoes. Soil OM may help retain soil Se, and prevent it from leaching. Mbuyu in particular had a lower soil OM, which is explained by a drier climate, higher temperature (faster microbial degradation of OM), and the absence of waterlogging (well-oxidised soils), which favours selenate over selenite. However, since selenate is less strongly adsorbed to the soil, it may be leached from the topsoil during heavy downpour. This explains the low plant-available Se in Mbuyu based on Se concentration in foods, as compared to locations with higher soil OM and plant available Se such as Ruiri and Njoune. Soil P concentration was another important influencing factor. Mbuyu largely differed from other

locations in soil P concentration. The presence of phosphate influences the extent to which Se is adsorbed by a soil and its availability in the soil solution (Christophersen et al. 2012). In this study, an increase in soil P was associated with an increase in Se concentration in bean, maize, and amaranths leaves. This is because soil phosphate causes desorption of selenite bound to soil minerals, as phosphate is bound more strongly to Fe and Al than is Se, and hence increasing Se availability for crop's uptake (Liu et al. 2004, Nakamaru et al. 2006, Eich-Greatorex et al. 2010). In our study, this was reflected by the soil's P to Fe + Al ratio, which was low in Mbuyu and high in Ruiri and Njoune.

Based on the actual dietary intake data, the diet of the rural population in the central Kenya highlands depends on a few cereal grains, beans, and potato/green banana. Intake of good Se sources such as fish, meat, and eggs is limited. Milk is routinely consumed but its contribution to dietary Se intake is restricted by its low Se concentration. This study therefore found that a low dietary diversity across the study locations resulted in an inadequate dietary Se intake. For instance, all children assessed in Mbuyu and Marimanti, and all women in Mbuyu, Marimanti, Mbeu, Kigucwha, and Ruiri had an average dietary Se intake below EARs (a risk of dietary Se deficiency of 100%). Notably, even women from locations with higher Se concentration in foods, such as in Ruiri and Njoune, had inadequate average dietary Se intake. Low dietary diversity is therefore an important factor contributing to inadequate dietary Se intake across the study locations. In general, 87% of children and 97% of women were at risk of dietary Se deficiency across the eight study locations. These results relate to previously reported low dietary Se supply of 23 - 35 µg capita^{-1} day^{-1} and mean estimated risk of dietary Se deficiency in Kenya at 91 - 100 % (Joy et al. 2014). The results also relate to the >80% risk of dietary Se deficiency in Malawi, based on plasma Se, blood GPx-3, and urinary Se to determine Se intake adequacy (Hurst et al. 2013). Specifically, 87% of children and 97% of women were at risk of dietary Se deficiency (Joy et al. 2015). In addition, this study observed lower dietary Se intakes among women in Mbuyu (14.1 µg d^{-1}), and that women were at a higher risk of dietary Se deficiency than children. This is because at times of limited food choices, the children's diet is given priority. Scarce and seasonal foodstuffs such as milk, eggs, fruits, indigenous vegetables, lentils and tubers are reserved for the children. Moreover, animal source foods such as milk and eggs are common ingredients for porridge that serves as a day time snack for children. The diet for the children is therefore generally more diversified compared to that of the rest of the household. This explains the 10% difference in the risk of dietary Se deficiency between the children and women.

In line with the dietary Se intake, children and women in Mbuyu had the lowest Se concentration in hair while their counterparts in Kiguchwa, Njoune, and Ruiri had the highest Se concentration in hair. The ranking of the dietary Se intake inadequacy (risk of Se deficiency) was therefore in line with the hair Se concentrations' findings, with the vast majority of the children and women's hair Se concentration (92% and 94% respectively) being below the reference interval. Hair Se concentration is related to long-term Se intake (Miekeley et al. 1998). Reference values for human

hair Se concentration have been reported in literature to be 0.77 mg kg^{-1} based on hair samples of healthy 3 to 15 years old youngsters from several urban areas of Rome, Italy (Senofonte et al. 2000). A reference interval of 0.7±0.1 mg kg^{-1} has also been calculated based on human scalp hair Se concentration from a group of 1091 individuals of Rio de Janeiro, Brazil (Miekeley et al. 1998). This is in line with reference median Se concentration of 0.79 mg kg^{-1} and a range of 0.48 to 1.84 mg kg^{-1} according to ALS Scandinavia AB laboratory – Stockholm, Sweden (Axelsson et al. 2001), and a range of 0.40 to 2.00 mg kg^{-1} reported by Biolab Medical Unit – London, UK (Austin and Soloway 2012). In this study, an increase in dietary Se intake from maize grains was associated with an increase in Se concentration in hair among women. Selenium in hair is therefore a sensitive and reliable indicator for dietary Se intake, as also reported in literature (IAEA 1994). This finding is important especially in limited resource settings where more accurate biomarkers such as serum Se cannot be measured. It is also important when targeting under-5 year old children, whereby collection of blood samples is ethically challenging. Miekeley et al. (1998) reported that Se level in tissues seems to be affected more easily and more generally by its abundance in the diet. This behaviour points to a less efficient homeostatic control and thus, the relations between Se content in the diet and that in the hair may be less disturbed by regulations than in other elements.

The low dietary Se intakes of <20 µg day^{-1} for instance in Mbuyu among women, indicated a high risk of dietary Se deficiency and geomedical problems among the local population. In addition, the inadequate dietary Se intakes of <40 µg day^{-1} among women across the study locations may pose an increased risk of oxidative stress-related diseases such as cancer and cardiovascular diseases, or progression of viral infection due to suppressed immune function (Rayman 2000, Fairweather-Tait et al. 2011). In a past study conducted in the coastal region of Kenya, Se deficiency was associated with higher likelihood of genital mucosal shedding of HIV-1 infected cells, increasing the infectiousness of women with HIV-1 (Baeten et al. 2001). Moreover, in child growth and development, inadequate Se intake during pregnancy poses a risk of impaired intrauterine growth retardation caused by oxidative stress (Biri et al. 2007). These past findings confirm Se deficiency as a potential important public health issue in Kenya, and SSA in general.

In conclusion, low Se concentration in foods contribute to the inadequate dietary Se intake in locations at a high risk of Se deficiency such as in Mbuyu, besides a low diversified diet. However in other study locations at a lower risk, a low diversified diet could be the main limiting factor in achieving an adequate dietary Se intake. Due to the limitation by the low sample sizes at the study location level that underpowered this study, the study calls for well powered studies with an adequate number of clusters in order to confirm these findings. In addition, the dietary intake data based on a single 24-hour recall is unable to describe usual dietary intakes of foods and Se. The study lacked data on the impact of seasonal variation on the risk of dietary Se deficiency. As a consequence, the study calls for additional studies based on multiple non-consecutive 24-hour recalls on the same individual in order to capture daily variability, increase the quality control,

minimizing errors, and maximizing reliability. Moreover, dietary intake data that capture seasonal variability is also needed.

A diversified diet that includes animal foods such as meat, eggs, and fish, and/or agronomic biofortification, which employs the use of fertilisers containing the mineral elements lacking in human diets, are potential interventional measures in the study locations at high risk of dietary Se deficiency (Ros et al. 2016). In addition, re-establishing agricultural ecosystems that are closer to the original natural ecosystem is the ultimate long-term solution for top soil's nutrient elements deficiencies (Christophersen et al. 2012). Soil nutrient deficiencies in rural subsistence farms mostly result from excessive disruption of natural systems of local biogeochemical recycling and retention through soil erosion and leaching. Improvements could be achieved through terracing and agroforestry, such as the introduction of Moringa oleifera tree, which is capable of concentrating Se in its edible leaves (consumed as vegetable/salad), compared to food crops grown on the same soil (Diriba et al. 2017a, Diriba et al. 2017b, Gashu et al. 2016).

CHAPTER 4: ASSOCIATION OF LOW DIETARY DIVERSITY WITH THE RISK OF DIETARY SELENIUM DEFICIENCY IN RURAL KENYA

4.1. Introduction

At present, over 820 million people are undernourished and an estimated two billion suffer from micronutrient deficiencies globally, in particular vulnerable population groups such as women and children (FAO 2018). Adequate amounts and a diversity of foods need to be consumed on a regular basis in order to achieve a balanced diet for good health and nutrition (Ruel 2003, Arimond et al. 2010). Nevertheless, securing adequate food that is healthy, safe and of high nutritional quality for all and at all times remains a global challenge (Willett et al. 2019). Typical diets across many low- and middle-income countries (LMIC) fall below quantity, quality and diversity requirements for healthy and nutritious diets (Zotor et al. 2015). As a consequence, malnutrition persists as a major public health concern in LMIC. In Africa, undernourishment affects 21% of the population. The situation is particularly pressing in sub-Saharan Africa (SSA) where an estimated 23% of the population suffers from chronic food deprivation (FAO et al. 2018). This has contributed to an unacceptably high prevalence of micronutrients deficiencies in the region. To illustrate, the East African region has the highest risk of deficiency for calcium (69%), zinc (75%), selenium (52%), and iodine (26%).

Inadequate dietary Se intake is therefore a widespread problem across SSA (Foster 2003), with Kenya in particular having a high risk of dietary Se deficiency ranging between 90-100% (Joy et al. 2014). This phenomenon is due - in part - to the nature of the food system, which is compounded with climate change, ecosystem and biodiversity pressures, population growth, urbanization, social conflict, and extreme poverty (FAO 2016). Moreover, national food security in many SSA countries relies on a narrow range of staple crops - especially maize - which are often produced by small-scale farmers under rainfed conditions. Hence, nutrition security and diet diversification are highly vulnerable to climate variability and extreme weather (Luckett et al. 2015). Furthermore, rural diets in LMIC are predominantly based on cereals and legumes or starchy roots and tubers. The consumption of fish, meat, and poultry, which are rich sources of Se, is usually limited due to economic, cultural, and religious constraints (Gibson and Hotz 2001, WHO 2004). Since Se is an essential nutrient for animal life, animal-source foods are richer sources of dietary Se than plant-based foods. To illustrate, Se concentration ranges from 0.08-0.73 mg kg^{-1} in meat, 0.11-0.97 mg kg^{-1} in marine fish, and 0.18-0.68 mg kg^{-1} in freshwater fish (WHO 2004). In comparison, Se concentration in cereal grains ranges from 0.01-0.55 mg kg^{-1} (Fairweather-Tait et al. 2010), and in fruits and vegetables from 0.001-0.022 mg kg^{-1} only, due to the low protein content coupled with a high water content (Fordyce 2007). In general, the Se concentration in food depends on the soil where crops are grown and the feeds for the livestock are raised on (Haug et al. 2007).

Selenium deficiency becomes evident at intakes below 30 µg day^{-1} (Yang and Xia 1995). The estimated dietary Se intake ranges between 30-50 µg day^{-1} in European countries (Fairweather-Tait et al. 2011), whereas it ranges between 23-35 µg day^{-1} in SSA countries (Joy et al. 2014). In addition, SSA countries have low per capita dietary Se availability of 27-45 µg $capita^{-1}$ day^{-1} with

risks of dietary Se inadequacy ranging from 26-75 % (Hurst et al. 2013). To illustrate, Chilimba et al. (2011) reported widespread sub-optimal dietary Se intakes between 20-30 µg day^{-1} in Malawi. Moreover, this thesis (Chapter 3) reported low average dietary Se intakes of 7.6 µg day^{-1} among children under 5 and 14.4 µg day^{-1} among women in Kenya. Selenium deficiency has been reported as particularly prevalent in geographical regions characterized by low soil Se availability (Mistry et al. 2012) and over-reliance on a narrow range of staple foods produced on these soils (Foster 2003), as is the case for subsistence farming households in SSA.

The above highlights the importance of between and within food group consumption to attain daily Se requirements. However, dietary diversity remains a challenge in food insecure countries and regions characterized by a high dependence on subsistence farming based on few staple cereal, legume, and tuber crops (Jones 2017). In addition, agricultural biodiversity which comprises a variety of cultivated plants and animals, indigenous foods and other products gathered by rural populations within traditional subsistence systems has been adversely affected by the degradation of ecosystems and climate change (Jarvis et al. 2006). As a consequence, the contribution of plant and animal biodiversity to food and nutrition security has drastically diminished in the last decades (Khoury et al. 2014). The resulting food insecurity coupled with a precarious nutrition transition has led to simplified and uniform diets, characterized by reduced consumption of nutrient-dense foods. The reliance on insufficiently diversified diets - based on energy-dense, nutrient-poor foods - exposes rural populations to Se deficiency and diet-related non-communicable diseases. Moreover, monotonous diets undermine the adaptive resilience inherent to biodiversity (Popkin 2002, Johns and Eryzaguirre 2006).

Food biodiversity and dietary diversity varies across agro-ecological zones (Lachat et al. 2018, Gitagia et al. 2019). Therefore, this chapter analyses food biodiversity and dietary diversity in nine study locations in rural Kenya; eight of the locations are located in the Central Highlands and one location in the Lake Basin. The study examines how food biodiversity and dietary diversity infer on the risk of dietary Se deficiency among children and women. Food biodiversity and food-group dietary diversity indicators were constructed to evaluate the associations between diet diversification and dietary Se intake, adequacy, and status in the various study locations. Moreover, hair Se concentration of the target population groups was analysed to explore the actual Se status. Thus, the study contributes to the literature by elucidating the importance of food biodiversity and dietary diversity on the prevalence of Se deficiency.

4.2. Context setting and method

4.2.1. Regional characteristics

Selenium concentration in food depends on the agricultural soils where crops are cultivated. Regional soil maps were therefore used to select study locations with different agricultural soils types (Jaetzold et al. 2006). Different soil types differ in geology and hence total soil Se concentration. It is the interaction with geochemical characteristics that influences the soil Se mobility and availability for plants' uptake (Dhillon and Dhillon 2000).

The study locations in Central highlands as described in the chapter 3, are characterized by relief precipitation and fertile soils due to volcanic parent materials. The region is intensively used for agriculture and is densely populated (Dijkshoorn et al. 2011), with a high volume of staple food production (International Livestock Research Institute 2015). Subsistence farmers produce and store little amounts of food, and have a low purchasing power for foods that are not locally produced (Jayne and Muyanga 2012). Rural households depend on a narrow range of staple grains produced from the same acreage of soil. Although milk, eggs, meat, vegetables, and fruits are locally produced, they are sold rather than consumed by the household. Furthermore, local communities traditionally raise cattle, goats, and sheep, but little meat is consumed in the Central Highlands. As a consequence, the intake of animal-source foods contributes only 0.5-10% of the total protein consumed within local diets, which is mainly based on cereal, legume, and tuber crops (Bwibo and Neumann 2003).

Kendu Bay, the only location that was studied in the Lake Basin is located on the shores of Lake Victoria. Fishing is an important economic activity in the surrounding villages, coupled with subsistence farming. For communities living along the shoreline, fish serves as both a nutritional safety net and a significant source of calories, protein, and micronutrients. It is the primary source of protein for local communities and it contributes the vast majority of dietary protein in areas near fisheries (Fiorella et al. 2014). Unlike the Central Highlands, Kendu Bay is characterised by conventional precipitation and unfertile soils, which mainly consist of sandy vertisol and ferrasol (Maobe et al. 1997). In the last decade, the Lake Basin has suffered from land degradation, low soil fertility and a decline in crop biodiversity (Maitima 2010). As a consequence, the region experiences low crop yields and reduced food and nutrient diversity (Walingo et al. 2009). Socioeconomic and environmental challenges in the Lake Basin have led to poverty and food insecurity (Balirwa et al. 2003), with most households having an inadequate protein intake (Bwibo and Neumann 2003). High commercialization of fishing marginalizes low resource families due to exploitation by middlemen, which compromises food security at household level (Geheb et al. 2008, Cheserek et al. 2012). In general, diets in the Central highland and in Kendu Bay are expected to diverge due to differences in climatic conditions, agrobiodiversity, and proximity to a natural fish source.

4.2.2. Target population and sample selection

The study population, sampling and randomization, inclusion and exclusion criteria, and ethical approval are as described in the Chapter 3. The data collection in the Lake Basin region was curtailed by first, political instability at the time of the data collection and second, a low participation rate by community members mainly due to the hair sampling for the individual Se status analysis. In the vast region of Western Kenya, communities strongly believe in evil magic, which is used to harm people, using objects closely related to the person e.g. hair, foot prints, or other articles belonging to the person (Kombo 2003). In the Central Highlands, few households still hold such traditional beliefs. In total, data for 184 children and 135 women were analysed in this chapter.

4.2.3. Assessment of dietary Se intake and Se status

Dietary intake assessment was conducted on women and mothers/guardian on behalf of their children as described in Chapter 2 section 2.2.1. Using dietary intake data collected from the 24HR, Dietary Species Richness (DSR) was calculated as an indicator of food biodiversity. DSR is the count of the number of distinct food species consumed by an individual per day (Lachat et al. 2018). Moreover, the Nutrient Adequacy Ratio (NAR) of Se and age-specific Diet Diversity Scores (DDS) were constructed as proxies of micronutrient adequacy of diets for women and children. The NAR for Se is the ratio of an individual's Se intake compared to the EAR for the subject's sex and age-bracket (Hatloy et al. 1998). For the age-specific DDS, ten food-groups were considered to construct Minimum Dietary Diversity for Women (MDD-W), including cereals/tubers/plantains, legumes/lentils, nuts/seeds, milk/daily products, meat/poultry/fish, eggs, dark green leafy vegetables, vitamin A-rich fruits and vegetables, other vegetables, and other fruits. Women reached MDD-W when the cut-off of ≥5 food-groups were consumed (Martin-Prevel et al. 2017). To construct Minimum Dietary Diversity (MDD) for children, the same food groups were considered, however, legumes/lentils were grouped with seeds and nuts, while other vegetables and fruits were pooled together, resulting in a total of seven food groups. Children reached MDD when the cut-off of ≥4 food groups (no minimum quantity) were consumed (WHO 2008).

In order to assess the Se concentration in foods consumed, various foods were sampled from the households. Selenium concentration in the food samples was then analysed following the procedure described in Chapter 2 section 2.3. Average dietary Se intake was estimated as described in Chapter 2 section 2.2.1. In addition, individual Se status was evaluated based on the mean hair Se concentration of the study population. Hair was sampled from children and women within the selected households as described in Chapter 2 section 2.2.2.

Statistical analysis was performed with SPSS – (IBM Corp. (2016). IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY) for the descriptive statistics of dietary intake data, and with STATA 15.1 for the dietary indicators. The normality of data was tested by the Shapiro-Wilk test. The descriptive statistics of the dietary intake data and the Se concentrations in food, hair, and soil

were based on untransformed data. The one-way ANOVA (Post Hoc Test) was used to analyse the differences between mean dietary Se intake, DSR, NAR, DDS and Se concentrations in foods, and hair between the study locations. Fixed effects models were applied to first evaluate the association between DSR and Se intake, NAR and hair Se status, and second to evaluate the association between DDS and Se intake.

4.3. Results

4.3.1. Food biodiversity and dietary diversity indicators

Food biodiversity, dietary diversity indicators, average dietary Se intake, and adequacy are presented in Table 13. There was no significant difference in DSR between the study locations for children (p-values > 0.05). For women, there was a significant difference in DSR between Kiaga and Mbeu (p-value = 0.005), Marimanti (p-value = 0.008), and Mbuyu (p-value = 0.002). On average, women in Kiaga consumed 10 unique food species in the preceding 24 hours while both women and children in other study locations consumed 7-8 food species.

There was no significant difference in DDS between the study locations for both women and children (p-values > 0.05). In all study locations, MDD-W was 4 food-groups while MDD ranged between 4-5 food-groups for children. Based on the recommended indicator-specific DDS cut-offs, the highest proportion of women who consumed ≥5 food groups was only 43% in Kiaga, followed by Kendu Bay (33%), and Mbeu (30%), while the lowest proportion was in Mbuyu (6%) and Kiguchwa (8%), as shown in Table 14. For children, the proportion that consumed ≥4 food groups was >70% in all study locations, with the lowest proportion reported in Marimanti (71%) and Kendu Bay (76%). This explains the significant associations between the DDS and Se intake among the children in five of the study locations, but only in Njoune for women (p-values < 0.05) as shown in Table 15. On average, for every additional food group consumed, daily dietary Se intake would increase by 2 µg day^{-1} for children. Since the average daily Se intake for children is 10 µg day^{-1}, an additional 7 µg day^{-1} is needed to achieve an EAR of at least 17 µg day^{-1} and hence, an additional 3-4 food groups are required. Moreover, <16% of women reached adequate dietary Se intake in all study locations while <26% reached adequate hair Se content. For children, <27% reached adequate dietary Se intake and hair Se content (Table 14).

For both women and children, there was a significant difference in average NAR (Se) between Mbuyu and the rest of the study locations (p-values < 0.05), except in Marimanti (p-value = 0.210). This is in line with the lowest proportion of women who achieved the DDS cut-offs in Mbuyu. This is explained by the significant difference in average Se intake of both women and children in Mbuyu from the other study locations (p-values < 0.05), except in Marimanti (p-value = 0.210). In addition, hair Se concentration among women and children in Mbuyu was significantly different from all other study locations (p-values <0.001). Mbuyu had the lowest Se concentration in hair for both women

and children (*Table* 13). There was no significant difference in average NAR (Se) or average Se intake between other study locations (p-values > 0.05). However, hair Se concentration among children in Kendu Bay was significantly different from other locations except in Mbeu, Kiaga, and Marimanti (p-values < 0.05). In general, hair Se concentration among women was significantly different across the study locations.

Table 16 reports the associations between DSR and average dietary Se intake, NAR, and hair Se status. The associations between DSR and dietary Se intake and NAR are positive and significant for both women and children in almost all of the study locations except in Kendu Bay. Generally, a one unit increase in DSR is associated with an increment in dietary Se intake, NAR (Se) (Figure 5), and in Se concentration in hair. In particular, for every additional intake of a food species, daily Se intake is likely to increase on average by 3 µg day^{-1} for women and by 1 µg day^{-1} for children. Since the average daily Se intake for women is 22 µg day^{-1}, an additional 23 µg day^{-1} is needed to achieve an EAR of at least 45 µg day^{-1} and hence, an additional 8 food species are required. For children, the average daily Se intake is 10 µg day^{-1}, an additional 7 µg day^{-1} is therefore needed to achieve an EAR of at least 17 µg day^{-1} and hence, an additional 7 food species are required. This is further demonstrated by Figure 5 which indicates that at population level, a daily consumption of more than ten food species may be required in order to achieve adequate Se intake.

Table 13: Dietary Se intake, Dietary Species Richness, Dietary Diversity Scores, Se Nutrient Adequacy Ratio, dietary energy intake, and hair Se concentrations for women and children on 8 locations in the central Kenya highlands and 1 location in the lake Victoria basin.

	Mean (SD)								
	Central Highlands								Lake Basin
Village	**Kiaga**	**Kibirichia**	**Kiguchwa**	**Marimanti**	**Mbeu**	**Mbuyu**	**Njoune**	**Ruiri**	**Kendu Bay**
Women									
n[1]	14		13	14	20	17	13	20	24
Se (µg)	25 (12)	-	27 (10)	17 (8)	19 (7)	8 (5)	29 (12)	22 (10)	26 (14)
DSR	10 (2)	-	7 (3)	7 (2)	7 (2)	7 (2)	7 (2)	7 (2)	8(2)
DDS[2]	4.4 (0.8)	-	3.8 (0.6)	3.9 (0.6)	4.2 (0.8)	3.8 (0.6)	4.0 (0.8)	4.0 (0.6)	4.0 (1.0)
NAR (Se)	0.545 (0.261)	-	0.598 (0.229)	0.381 (0.169)	0.418 (0.166)	0.176 (0.114)	0.647 (0.276)	0.479 (0.212)	0.589 (0.314)
Energy (kcal)	2556 (767)	-	2579 (595)	2180 (1112)	2412 (825)	1874 (767)	2443 (1210)	1983 (779)	2182 (933)
Hair Se status (n[1])	6	-	10	7	16	8	5	12	23
Hair Se (mg kg^{-1})	0.48 (0.08)	-	0.66 (0.09)	0.50 (0.05)	0.59 (0.09)	0.24 (0.05)	0.58 (0.09)	0.66 (0.16)	0.45 (0.09)
Children									
n[1]	14	27	9	14	25	19	16	31	29
Se (µg)	11 (4)	12 (6)	9 (4)	9 (5)	10 (4)	5 (2)	11 (7)	13 (6)	12 (7)
DSR	9 (2)	7 (2)	6 (2)	7 (2)	9 (2)	8 (2)	8 (4)	8 (2)	8 (2)
DDS[2]	4.4 (1.3)	4.4 (1.0)	4.3 (1.0)	4.0 (1.3)	4.7 (1.0)	4.9 (0.9)	4.4 (1.4)	4.4 (0.7)	4.1 (1.1)
NAR (Se)	0.572 (0.187)	0.614 (0.306)	0.459 (0.215)	0.454 (0.229)	0.514 (0.179)	0.255 (0.112)	0.567 (0.370)	0.638 (0.301)	0.610 (0.342)
Energy (kcal)	1416 (659)	1369 (538)	1128 (641)	586 (452)	1216 (736)	1002 (407)	1247 (900)	1130 (754)	1190 (701)
Hair Se status (n[1])	9	21	8	8	20	17	15	26	26
Hair Se (mg kg^{-1})	0.49 (0.06)	0.57 (0.15)	0.61 (0.09)	0.51 (0.05)	0.55 (0.07)	0.29 (0.05)	0.63 (0.10)	0.63 (0.16)	0.46 (0.11)

[1]Number of study locations. Note: Se = Selenium; DSR = Dietary Species Richness; DDS = Dietary Diversity Scores; NAR = Nutrient Adequacy Ratio; SD = standard deviation. -: Data not available

Table 14: Proportions (%) of women and children reaching adequate Se EAR, MDD-W/DDS, and cut-off for adequate hair Se status for women and children on 8 locations in the central Kenya highlands and 1 location in the lake Victoria basin.

	Proportion (%)								
	Central Highlands								Lake Basin
Village	**Kiaga**	**Kibirichia**	**Kiguchwa**	**Marimanti**	**Mbeu**	**Mbuyu**	**Njoune**	**Ruiri**	**Kendu Bay**
Women									
Se EAR	7	[1]-	0	0	0	0	15	0	13
MDD-W	43	-	8	14	30	6	23	15	33
Ref. Hair Se	0	-	15	0	10	0	0	25	4
Children									
Se EAR	0	19	11	0	0	0	25	26	21
MDD	86	85	89	71	92	90	81	90	76
Ref. Hair Se	0	11	11	0	0	0	25	26	3

Se EAR: Estimated Average Requirement of Se. MDD-W: Minimum Dietary Diversity for Women. MDD: Minimum Dietary Diversity. Ref. Hair Se: Reference value - Se hair content

Table 15: Significant linear regression equation coefficients (β) between Diet Diversity Score and Se intake on 8 locations in the central Kenya highlands and 1 location in the lake Victoria basin

	β (SE)								
	Central Highlands								Lake Basin
Village	**Kiaga**	**Kibirichia**	**Kiguchwa**	**Marimanti**	**Mbeu**	**Mbuyu**	**Njoune**	**Ruiri**	**Kendu Bay**
Women									
n	14	[1]-	13	14	20	17	13	20	24
DDS	4.4 (4.3)	-	8.6 (5.0)	6.2 (3.1)	3.5 (2.0)	2.1 (2.3)	8.9 (3.7)	2.9 (3.6)	0.6 (3.0)
Children									
n	14	27	9	14	25	19	16	31	29
DDS	1.7 (0.7)**	2.9 (1.1)**	2.3 (1.4)	1.8 (0.9)**	1.5 (0.7)**	0.8 (0.6)	2.4 (1.3)	3.4 (1.4)**	1.7 (1.1)

Note: SE = Standard Error; DDS = Diet Diversity Score. **Significant at the 5 percent level. [1]-: Data not available

Table 16: Significant linear regression equation coefficients (β) between between Dietary Species Richness and Se intake, Nutrient Adequacy Ratio and hair Se status for women and children

	β (SE)								
	Central Highlands								Lake Basin
Village	**Kiaga**	**Kibirichia**	**Kiguchwa**	**Marimanti**	**Mbeu**	**Mbuyu**	**Njoune**	**Ruiri**	**Kendu Bay**
Women									
n	14	[1]-	13	14	20	17	13	20	24
Se (µg)	3.2 (1.3)**	-	2.1 (0.9) **	2.2 (0.8)**	1.8 (0.7)**	1.0 (0.7)	4.1 (1.7)**	0.8 (1.4)	2.3 (1.6)
NAR (Se)	0.072 (0.029)**	-	0.048 (0.19)**	0.049 (0.017)**	0.040 (0.016)**	0.021 (0.015)	0.091 (0.038)**	0.018 (0.030)	0.051 (0.035)
Hair Se status (n)	6	-	10	7	16	8	5	12	23
Hair Se (mg kg^{-1})	0.010 (0.017)	-	0.013 (0.016)	0.019 (0.009)	0.011 (0.011)	0.007 (0.009)	0.016 (0.015)	0.002 (0.032)	0.008 (0.010)
Children									
n	14	27	9	14	25	19	16	31	29
Se (µg)	1.0 (0.3)**	1.4 (0.4)**	0.9 (0.6)	1.3 (0.4)**	0.4 (0.3)	0.5 (0.2)**	1.5 (0.4)**	1.6 (0.5)**	0.7 (0.6)
NAR (Se)	0.053 (0.016)**	0.071 (0.022)**	0.046 (0.029)	0.065 (0.020)**	0.023 (0.015)	0.026 (0.11)**	0.074 (0.20)**	0.078 (0.025)**	0.035 (0.29)
Hair Se status (n)	9	21	8	8	20	17	15	26	26
Hair Se (mg kg^{-1})	0.007 (0.011)	0.001 (0.014)	0.014 (0.013)	0.001 (0.008)	0.022 (0.005)**	0.006 (0.006)	0.016 (0.007)**	0.018 (0.016)	0.001 (0.009)

Note: SE = Standard Error; Se = Selenium; NAR = Nutrient Adequacy Ratio. **Significant at the 5 percent level. [1]-: Data not available

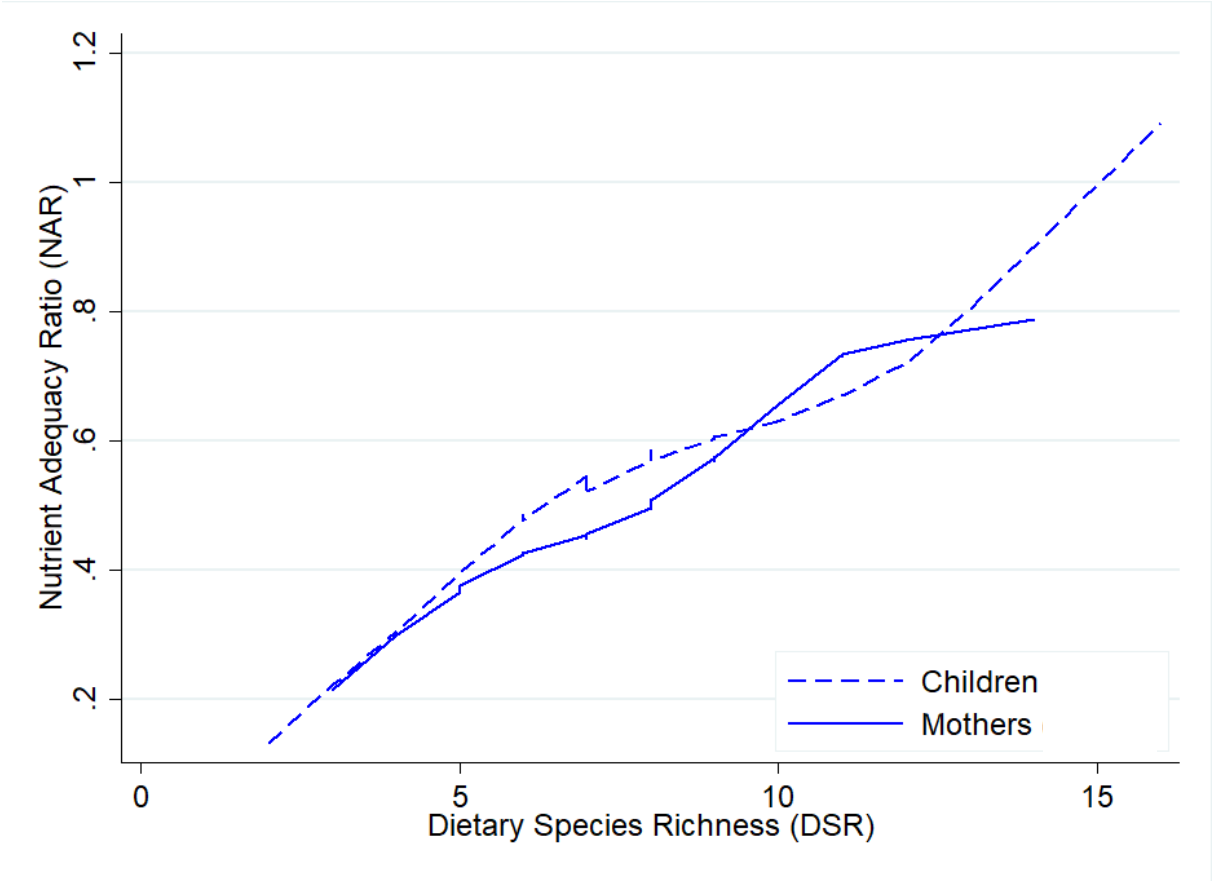

Figure 5: Association of DSR with NAR of Se for mothers (n=135) and children (n=184)

4.4. Discussion

This study aimed at establishing the associations between indicators of dietary diversity and dietary Se intake in rural Kenya. Although the study locations were characterized by distinct dietary compositions, no significant difference in food biodiversity was found except in Kiaga. On average, 7 to 8 food species dominated the diets of both women and children; however, 10 food species were reported in Kiaga. This explains the similarity in dietary diversity across the study locations, with the women having consumed only 4 foods while the children consumed 4 to 5 foods. Among the study locations in the Central Highlands, Mbeu, Marimanti, and Mbuyu are the most arid and depend on rain-fed agriculture. Exceptionally in Kiaga, subsistence farmers practice both rain-fed and irrigation-based agriculture. This explains the availability of more food species in Kiaga such as cowpeas, pigeon peas, sweet potatoes, spinach, and indigenous vegetables that are not cultivated in arid areas under rain-fed agriculture. The other study locations receive considerably adequate rainfall throughout the year.

Although there was no significant difference in DDS between the study locations for both women and children, we found the highest proportion of women (43%) who achieved the recommended DDS cut-off of ≥5 food groups in Kiaga, followed by Kendu Bay (33%). While irrigation-based

agriculture increases food species in Kiaga, fish is mainly consumed by communities living on the shores of Lake Victoria such as in Kendu Bay. The proportion of fish consumption in Kendu Bay higher than intake of meat in the Central Highlands. Generally, a higher proportion of children achieved the recommended DDS cut-off compared to women. This can be explained on the one hand by the fact that women and children in rural areas depend on the same staple foods, of which a dietary diversity score of 4-5 food is within the cut-off for children. On the other hand, due to limited food resources in the rural areas, the children's diet is given priority in the households especially during low food seasons. This further explains the higher dietary Se adequacy among the children, as compared to women.

Overall, this research found inadequate Se intake across the study locations and hence a high risk of dietary Se deficiency. This is evident by a positive and significant associations between food biodiversity and dietary Se intake, adequacy, and Se status for both women and children. Low food biodiversity and food-group diversity, in addition to low Se concentrations in local foods, as reported in Chapter 3, are therefore the main contributing factors to the high prevalence of dietary Se inadequacy. Low food biodiversity is explained by unsustainable agriculture methods, with maize serving as both cash and food crop (Makalle et al. 2008). According to Walingo et al. (2009), unsustainable food consumption patterns are a result of low crop yields. Deforestation leads to widespread conspicuous land degradation (Makalle et al. 2008), and consequently a warmer and a drier climate and poor soil productivity due to nutrient depletion (Maitima et al. 2004). In Kendu Bay in particular, it is generally expected that the proximity of the Lake Basin to the fisheries results in higher fish consumption. However, and as explained by Geheb et al. (2008), fishing in the Lake Victoria is highly commercialized which marginalizes low resource families, and consequently, compromises fish consumption at household level (Cheserek et al. 2012).

This study further found that a one unit increase in DSR is likely to result in an increment in dietary Se intake, NAR (Se), and in Se concentration in hair. In order to achieve adequate Se intake at the population level, an additional 8 and 7 food species would be required for women and children respectively, or approximately an additional 3-4 food groups per day. Since the target population already consumes on average 7 to 8 food species per day, an additional 7 to 8 species will be difficult to achieve. Furthermore, less than 44% of women consumed ≥5 food groups, and hence it will be difficult to consume an additional 3-4 food groups per day. In general, these objectives are difficult to reach considering the challenges that the rural population faces in terms of low income, dependence of rain-fed agriculture, poor agricultural practices and hence low food production, and climate change. About half of the Kenyan population are unable to afford sufficient food to meet recommended daily requirements, mainly in rural areas. The poverty trend in Kenya indicates a stagnant aggregate on average welfare gains for the poorest quintile (World Bank 2009). Access to high quality nutritious foods therefore remains a major challenge among rural population (Fanzo 2012). In addition, large family size and illiteracy contribute to poor diet and nutrition (Bain et al., 2013). Under the prevailing circumstances, dietary diversification strategy alone will hardly achieve

adequate Se intake. Improvement of Se intake outcomes will therefore need besides efforts to diversify the diet of the rural population, other population-based approaches involving food biofortification, along with food security, and nutrition education.

In conclusion, diets in both regions are characterized by a low food biodiversity and dietary diversity, particularly among women, resulting in inadequate dietary Se intakes and a high risk for dietary Se deficiency. Since food biodiversity is positively and significantly associated to dietary Se intake, adequacy, and Se status, dietary diversification has intrinsic potential to improve dietary Se intake to adequate intake levels. The challenges highlighted above withstanding, there is a need to address Se deficiency through a combination of agricultural- and nutritional-focused campaigns and policy measures. This includes a combined population-based approach that includes diet diversification, food biofortification, and improved soil management. The limitations of the study include the observational design which is unable to infer causality of findings and differences in the number of sample sizes per study location which introduce bias for regional comparisons. In addition, assessment of individual Se status based on hair samples is not as sensitive and accurate as other biomarkers such as serum Se and GPx-3 measurements. Moreover, the dietary data based on a single 24-hour recall lacked information on usual intakes, seasonal variability and hence, the study calls for well powered studies with adequate sample sizes and conducted at different seasons throughout the year in order to confirm these findings.

CHAPTER 5: AGRONOMIC BIOFORTIFICATION OF MAIZE AND BEANS IN KENYA THROUGH SELENIUM FERTILIZATION

5.1. Introduction

Multiple micronutrient deficiency remains an important health issue in developing countries. The deficiencies are mainly linked to poor access to high quality nutritious foods and a poorly diversified diet, based on a few staple foods that supply low amounts of micronutrients (Kennedy et al. 2007). Most rural households depend on a small parcel of land for subsistence farming (Jayne and Muyanga 2012), and hence, inherent soil micronutrients deficiencies are exacerbated by continuous cropping without nutrient replenishment (MoA 2013). Over time, dietary micronutrient deficiencies set in among the rural population, leading to adverse lifelong consequences on health, productivity, and mental impairment (Micronutrient Initiative 2009).

An assessment of Se status in the Central Kenya Highlands revealed Se deficient agricultural soils with a median total Se concentration of 0.439 mg kg^{-1}. The study also found low Se concentration in foodstuffs grown on these soils as well as average dietary Se intake of 14.8 and 32.6 µg day^{-1} among under-5 children and women respectively, which is below daily requirements. Consequently, an estimated 87% of children and 97% of women are at risk of dietary Se deficiency in the Central Kenya Highlands. Based on an estimated dietary intake survey conducted in the region, a diet based on local cereal grains of low Se concentration with limited or no intake of good Se sources such as meat and fish, is one of the major underlying factors of Se deficiency.

As discussed in Chapter 4, a high dietary diversity can achieve dietary Se intake requirements. Improved nutrition however requires not only a higher dietary quality and diversity, but also the constant access to the foods. Townsend (2015) highlights improvement of nutritional outcomes through a combination of support as one of the key elements for action to end hunger and poverty. This includes: raised income to allow families to invest in more and higher nutritious food consumption; expand coverage of nutrition-specific investments such as nutrition supplementation for children and pregnant women; refocused investments to make them more nutrition-sensitive such as nutrition-sensitive agriculture; and ensuring food availability and stability, among others. According to the World Bank (2009), about half of the Kenyan population mainly in rural areas, are unable to afford sufficient and diversified diet to meet recommended daily requirements. Since the vast majority of the rural population in Kenya are subsistence farmers, diversifying their food production can be a useful approach to improve dietary diversity. In this regard, Sibhatu et al. (2015) conducted a study on the link between food production and consumption diversity in Kenya, Ethiopia, Malawi, and Indonesia. They reported that on-farm production diversity is positively associated with dietary diversity, however, when production diversity is high, the association is not significant or even turns negative. Moreover, access to the market had larger positive effects on dietary diversity than those of increased production diversity, while market transactions reduced the role of farm diversity for household nutrition. These results indicated that increasing on-farm diversity is not always the most effective way to improve dietary diversity in smallholder households and should not be considered a goal in itself, and hence the need to make agriculture and food

systems more nutrition-sensitive. This reiterates the need for a combined support above as recommended by Townsend (2015). In addition other factors to consider for a diversified food production in the rural areas include the nutrients depleted soils due to continuous cropping without nutrient replenishment, high farm-inputs costs, climate change, and illiteracy (Bain et al. 2013).

Existing national micronutrient deficiency intervention measures in Kenya include fortification of processed foods and supplementation (MoH 2017). However, rural dwellers have low access to commercial fortified foods and nutritional supplements. More feasible strategies such as agronomic biofortification are therefore needed, to complement the existing ones in reaching rural communities whose diets depend mainly on subsistence farming (Gibson and Hotz 2001). Agronomic biofortification consists of applying fertilizers of mineral elements lacking in the diet in order to increase their concentrations in crops (Lavu et al. 2013), through soil or foliar Se fertilizers application (Ros et al. 2016). The strategy takes advantage of the consistent daily consumption of large amounts of local staple foods in low-income households (Lyons et al. 2004a). However, it should be noted that Se, in contrast to e.g. Zn and Fe, is considered a non-essential element for crop's growth and has no effect on crop's yield (Chilimba et al. 2012, Wang et al. 2013, and Mao et al. 2014). Farmers would therefore not benefit economically from Se fertilization through an increased yield and hence, governmental incentives and regulations encouraging the use of Se-enriched fertilizers are needed (Graham et al. 2007).

The fate of Se fertilizer is fundamentally dependent on the behavior of Se in the soils. Selenium occurs naturally in all types of soils with global quantities ranging from 0.01 to 2.0 mg kg^{-1} (Rayman 2008). The soil Se deficiency threshold in agricultural soils is reported to range from 0.1 to 0.6 mg kg^{-1} (Fordyce 2013). Soil types vary in geochemical characteristics, which determine the chemical forms of Se, and consequently influence its interaction, uptake by plants, and concentration in edible plant's parts (De Temmerman et al. 2014). Selenium uptake by plants, translocation, and distribution depend upon the plant's accumulation capacity, stage of growth, Se form or concentration, and soil's physiological conditions (Zhao et al. 2005). The behaviour of trace elements in the environment is determined by their specific physiological forms rather than their total concentration (Du Laing 2010) and hence, the total soil Se concentration does not indicate its bioavailability to crops (Broadley et al. 2006).

The rural population in the Central Kenya Highlands depends on a few staple cereal grains. Since agronomic biofortification can increase the concentration of mineral elements often lacking in human diets, in particular Se, Zn, and I (White and Broadley 2009), the local staple crops such as maize and legumes can be prime candidates for micronutrients biofortification. For example, Mao et al. (2014) biofortified winter wheat, maize, soybean, potato, canola, and cabbage with Se, Zn, and I fertilizers, both independently, and in combination. The majority of the malnourished rural population exhibits multiple micronutrient deficiencies (MoH et al. 2013), and thus, a multi-mineral agronomic biofortification of staple crops can be explored. Therefore, this study investigated staple crops' response to a combined multi-mineral agronomic biofortification.

This study investigated the response of staple crops to Se fertilizer in the Central Kenya Highlands. The research aimed to apply selenate fertilizer to maize and bean crops using both soil and foliar fertilizer application techniques, and studied its effects on Se concentration in the grains. The biofortification experiment was conducted at locations identified in chapter 3 to be at risk of dietary Se deficiency, to evaluate the crops' response to Se fertilizer application treatments, and compare them to a control. Soil geochemical characteristics of control experimental plots were analysed to examine the soil's status before biofortification. Due to depletion of soil macronutrients as a result of continuous cropping in rural subsistence farming without nutrient replenishment, the study evaluated also the effect of adding phosphorus (P) and nitrogen (N) fertilizers to the soil Se fertilizer application, on Se concentration in grains. The study then investigated the effect of combining Se fertilizer with Zn and I fertilizers on Se concentration in grains through foliar fertilizer application.

5.2. Materials and methods

5.2.1. Target study locations

The Central Kenya Highlands are characterized by a high staple food production (Institute of Agricultural Policy 2013), with the local diet mainly relying on local staple cereal grains. The region also contains a wide range of agricultural soil types that differ in their geology, relief, and climate (Giachene and Kimaru 2003), and therefore likely vary in Se concentration and bioavailability for uptake by crops. The study accounted for this heterogeneity in soil types by conducting a Se biofortification trial at three locations: Mbuyu, Mbeu, and Kiaga characterized by chromo-luvic phaeozems, humic acrisols and luvisols, and verto-eutric nitisols, respectively. Figure 6 represents the study area map and target soil types. The study locations are characterized by Se deficient agricultural soils, low Se concentration in local foodstuffs, and sub-optimal dietary Se intake among the local population. Total soil Se concentration varies on average from 0.372 mg kg^{-1} in Mbeu to 0.392 in Kiaga, and 0.466 mg kg^{-1} in Mbuyu. The low soil Se results in low Se concentration in local foodstuffs and consequently, inadequate average dietary Se intake.

5.2.2. Target study crops

Based on estimated dietary intake assessment data from chapter 3 among under-5 children and women at the study locations, 74% of children and 84% of women reported to have consumed maize, while 57% under-5 children and 75% women consumed beans. These staple foods therefore constitute a dominant portion of the daily local standard diet for the local population. Besides, maize and beans crops are grown at all three study locations. The crops are therefore relevant candidates for the biofortification experiment, as compared to other common staple foods such as potatoes, rice, and wheat, which are not locally produced at all study locations.

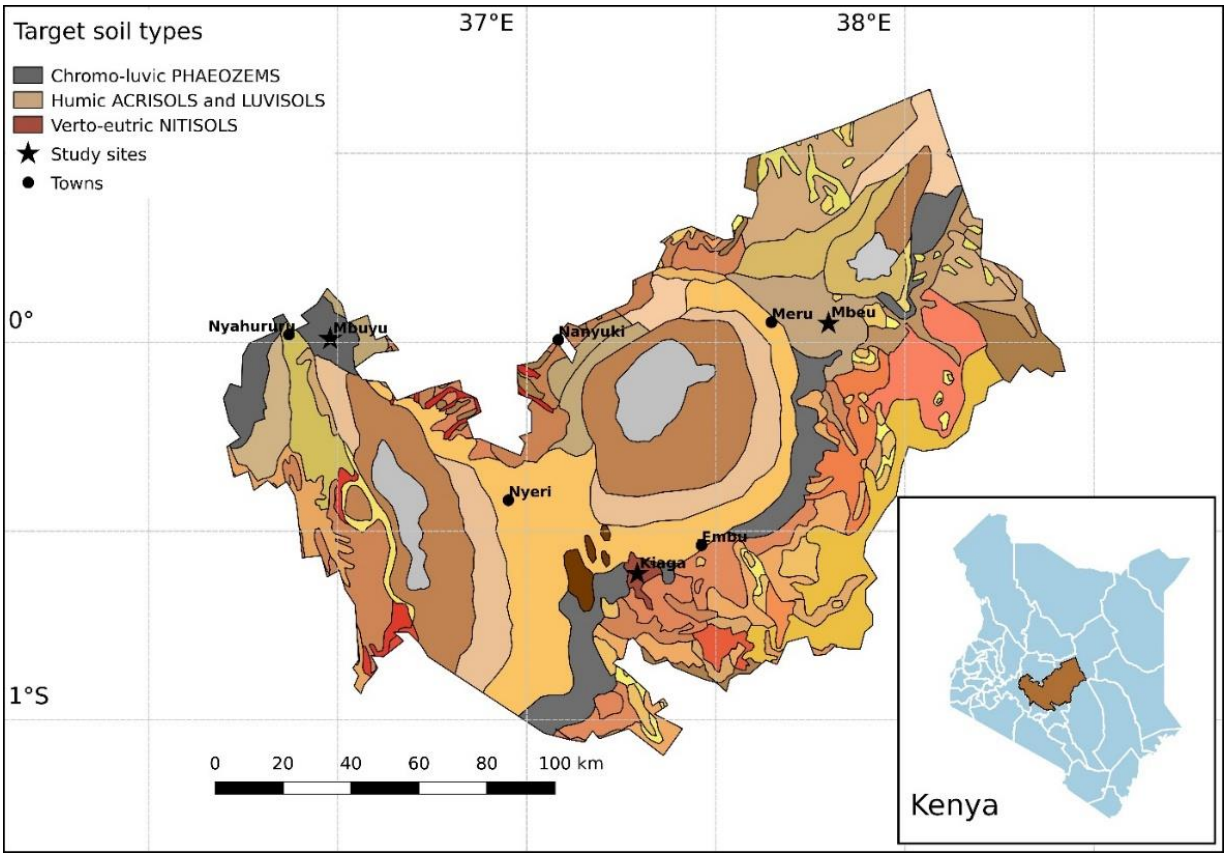

Figure 6: Selenium biofortification trial study locations in Central Kenya Highlands

5.2.3. Method

- Soil and foliar Se fertilizer application

Based on the results from the Se status survey at the study locations (Chapter 3), three farms with total soil Se concentration below the Se deficiency threshold were selected at each study location. The farms were ploughed and harrowed between March 15 and 30, 2016 in preparation for the experiment studying the effects of sodium selenate (Na_2SeO_4) at 0, 5, 10, and 20 g ha^{-1} treatments on Se concentration in grains. Each treatment was conducted on a 4 m x 2 m experimental plot in duplicate. Three Se fertilizer application experiments were conducted: soil Se fertilizer application, soil Se fertilizer application plus diammonium phosphate ($(NH_4)_2HPO_4$) and urea (CH_4N_2O), and foliar Se fertilizer application. The choice of Se trial doses was based on the current recommendation systems for Se that include Se doses of ~10 g Se ha^{-1} for arable systems (Ros et al. 2016). Our study further tested half of this recommended dose (5 g Se ha^{-1}) as well as double the dose (20 g Se ha^{-1}). On each 4 m x 2 m experimental plot, three rows of 'sowing holes' of 3 cm deep were prepared. The spacing between rows was equal to 75 cm and the spacing between holes was equal to 30 cm. Through practice using plain water and a knapsack sprayer, the application rate of the Se fertilizer drench and the amount (3 L) required to evenly spread it in the 4m x 2m plot was determined.

Seeding was done at the start of early rains from April 4 to 9, 2016. In the soil application treatment, Se fertilizer was evenly sprayed on the top soil before seeding, with the applicant wearing protective clothing. The fertilizer treatments were randomly allocated to the plots in duplicate. In total, there were 36 (4 m x 2 m) experimental plots for the soil application per trial farm. Seed varieties that are locally recommended per agro-ecological region were sowed. For maize, variety KH 600-15A was used in Mbuyu and KH 500-33A in Mbeu and Kiaga. For beans, variety K132 was used in Mbuyu while GLP24 was used in Mbeu and Kiaga. Following the local intercropping practice, two rows of maize were sowed on the outer two rows of the experimental plots and one row of beans in the middle. Two seeds were sowed per hole. For the soil Se application plus P and N experiment, a base application of 60 kg P ha^{-1} was conducted before sowing, and 200 kg N ha^{-1} was top dressed during crop's stem elongation. The application rate for P and N fertilizers was based on recommended doses for cereal crops (Ros et al. 2016). In the foliar application technique, fertilizer treatments were also randomly allocated to the plots in duplicate and the Se fertilizer was evenly sprayed on crop leaves during the stem elongation stage (knee-high) for maize, and just before flowering for beans. For the full factorial combination of sodium selenate (Na_2SeO_4) with zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) and potassium iodate (KIO_3), all possible combinations were applied i.e. 4*Se (0, 5, 10, 20) g Se ha^{-1}, 3*Zn (0, 2, 4) kg Zn ha^{-1}, and 3*I (0, 5, 10) g I ha^{-1}. The combined fertilizer drench was then sprayed evenly on the crop leaves. In total, there were 72 plots for the foliar application per trial farm.

- Sampling, sample preparation, and chemical analysis

Bean grains were sampled after the crops had reached physiological maturity as from July 25 to 30, 2016. The 4-month maize variety in Mbeu and Kiaga was sampled within the week of August 8 to13, 2016, while the 6-month maize variety in Mbuyu was sampled from October 3 to 8, 2016. From each experimental plot, a composite sample of grains was prepared from all the crops per plot. The samples were then winnowed to remove chaff, dried in dry air ovens at 70 0C, and grounded into powder using a washable dry mill blender to avoid cross contamination. Before the experiments, soil was sampled from experimental plots using a similar procedure as described in section 2.2 Chemical analysis for the maize, bean, and soil samples was conducted as described in section 2.3.

- *Statistical analysis*

Statistical analysis was performed with SPSS - IBM Corp. (2016). IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY. Normal distribution of data was verified by the Shapiro-Wilk Test. Data that was not normally distributed were log10 transformed before further analysis. Difference between mean Se concentrations in grains across study locations was tested using one-way ANOVA (Post Hoc Test). Linear regression models were derived to determine the relation between Se concentrations in grains and Se fertilizer application dose. Two-way and three-way factorial ANOVA explored for the effect of combining Se fertilizer with Zn and I fertilizers, on Se concentration in grains. A statistical confidence level of 95% was applied.

5.3. Results

5.3.1. Soil status of experimental farms

The mean total soil Se concentration was 0.339, 0.355, and 0.356 mg kg^{-1} in Mbeu, Kiaga, and Mbuyu, respectively (Table 17). There was no significant difference in mean total soil Se concentration between experimental plots across the three study locations. However, mean extractable soil Se in Mbeu at 0.005 mg kg^{-1} significantly varied from Kiaga at 0.006 mg kg^{-1} (p-value = 0.046) and Mbuyu at 0.007 mg kg^{-1} (p-value = 0.007). The geochemical characteristics did not help to explain Se concentration in grains as no significant correlations were found between geochemical characteristics and Se concentrations in the grains in the control, except for $CaCO_3$ (CC = 0.957, p-value = 0.04).

Table 17: Soil geochemical characteristics (mean with standard deviation (SD) at 3 locations in Kenya (Mbuyu, Mbeu and Kiaga)

	Mbuyu		**Mbeu**		**Kiaga**	
Parameter	Mean	*SD*	Mean	*SD*	Mean	*SD*
Total Se	0.356	*0.139*	0.339	*0.018*	0.355	*0.026*
Ext. Se[a]	0.007	*0.001*	0.005	*0.001*	0.006	*0.001*
OM[b] (%)	1.09	*0.23*	1.89	*0.08*	1.57	*0.11*
OC[c] (%)	0.63	*0.14*	1.10	*0.05*	0.91	*0.07*
$CaCO_3$ (%)	0.42	*0.05*	0.58	*0.03*	0.54	*0.02*
N (mg g^{-1})	2.12	*0.33*	1.90	*0.18*	1.67	*0.39*
pH H_2O	6.21	*0.26*	5.78	*0.17*	6.13	*0.18*
Mn (g kg^{-1})	1.63	*1.11*	4.26	*0.75*	3.04	*1.46*
Co	9.5	*4.8*	90	*6.1*	44	*1.6*
Cu	11.9	*4.9*	68	*10.8*	31	*9.0*
Al (g kg^{-1})	39	*16*	92	*8.0*	112	*7.0*
Fe (g kg^{-1})	45	*16*	119	*6.5*	123	*11*
Cr	22	*6.9*	179	*16*	43	*33*
Ni	20	*1.7*	188	*43*	35	*24*
Zn	81	*33*	172	*5.9*	123	*24*
Na	104	*21*	80	*8.9*	100	*10*
S	218	*59*	273	*29*	237	*50*
K	2474	*822*	926	*208*	1042	*331*
P	294	*74*	1915	*420*	3107	*1592*
Mg	1261	*219*	2874	*570*	2849	*531*
Ca	1756	*426*	1672	*673*	2218	*658*

[a]Ext. Se = Extractable soil Se concentration; [b]OM = Organic Matter, [c]OC = Organic Carbon; n = 4 per study location: each treatment was conducted in 4 experimental plots per study location.

5.3.2. Effect of soil Se fertilizer application on Se concentration in maize and beans grains

Compared to the control, application of Se fertilizer on the soil significantly increased Se concentration in maize and beans at all study locations (p-value < 0.001), as shown in Table 18 and Table 19. The increase in Se concentration was significantly higher in beans than in maize (p-value < 0.05). For each treatment, Se concentration in maize grains in Mbuyu was significantly

lower compared to Mbeu and Kiaga (p-value < 0.001)[1], as shown in Table 18. The effect of 20 g Se ha^{-1} on maize was significantly lower in Mbuyu compared to the other 2 locations (p-value < 0.05). However, the effect of 5 and 10 g Se ha^{-1} treatments in maize did not vary across the locations. For beans, no significant variation of Se concentration was observed in the control and 5 Se g ha^{-1} treatment. However, Se concentration in beans significantly varied between Mbuyu and Mbeu for the 10 Se g ha^{-1} (p-value = 0.003) and 20 Se g ha^{-1} (p-value = 0.03) treatments (Table 18)).

Combining soil Se fertilizer application with P and N fertilizers had a positive effect on Se concentration in both maize and beans (Table 18 and Table 19). Compared to the soil Se application without P and N fertilizers, Se concentration in maize increased on average across the study locations by 21% in maize and 19% in beans. The effect of P and N fertilizers was generally higher in Mbeu and Kiaga than in Mbuyu. The Se concentration in maize grains was significantly higher when P and N fertilizers were applied in Mbuyu for the 20 g ha^{-1} treatment (p-value = 0.035), Mbeu for the 10 g ha^{-1} treatment (p-value = 0.0004) and Kiaga for the 10 and 20 g ha^{-1} treatments (p-values = 0.01 and 0.013).

5.3.3. Effect of Foliar Se fertilizer application on Se concentration in maize and beans grains

The foliar fertilizer application significantly increased Se concentration in maize and beans compared to the control (p-value < 0.001), as shown in Table 18 and Table 19, and the increase was again significantly higher in beans than in maize (p-value < 0.05). Selenium concentration in the maize grains varied significantly across the study locations for each foliar application treatment (p-values < 0.05), apart from the control between Mbeu and Kiaga (p-value = 0.24). In beans, there was no significantly difference in Se concentration for the control treatment (p-value = 0.36) between Mbuyu and Kiaga, while for the 5 Se g ha^{-1} treatment, variation was observed across all locations (p-value < 0.05). Concentrations in Mbuyu were significantly different from those in Mbeu and Kiaga for the 10 Se g ha^{-1} and 20 Se g ha^{-1} treatments (p-value < 0.001).

As observed for soil application, the effect of Se fertilizer on Se concentration in grains differed significantly in Mbuyu as compared to the other two locations, for the three treatments tested (p-values < 0.05). Moreover, for each Se fertilizer application dose, foliar fertilizer application resulted in a significantly higher Se concentration in grains as compared to soil fertilizer application (p-values < 0.05). On average across the study locations, foliar application was more effective than soil application.

[1] One-way ANOVA (Post Hoc Test) for maize between Mbeu and Kiaga: p-value = 0.377 (control), 0.979 (5 g Se ha^{-1}), 0.307 (10 g Se ha^{-1}), 0.784 (20 g Se ha^{-1})

Table 18: The effect of Se fertilizer application techniques and doses on Se concentration (mg kg^{-1}) in maize grains on 3 locations in Kenya (Mbuyu, Mbeu and Kiaga)

		Soil Se application			**Soil Se application + P & N**				**Foliar Se application**		
Location	*Se (g ha^{-1})*	*Mean*	*SD*	*Effect*[a]	*Mean*	*SD*	*[1]Effect*	*[b]Increase*	*Mean*	*SD*	*[1]Effect*
Mbuyu	Control	0.011	0.004	0	0.011	0.006	0	1	0.011	0.003	0
	5	0.021	0.004	0.010	0.022	0.005	0.011	6	0.032	0.006	0.021
	10	0.030	0.009	0.020	0.033	0.004	0.022	8	0.047	0.008	0.036
	20	0.037	0.007	0.027	0.064	0.007	0.054	72	0.197	0.008	0.186
Mbeu	Control	0.043	0.002	0	0.043	0.002	0	1	0.043	0.003	0
	5	0.053	0.014	0.010	0.083	0.014	0.040	57	0.187	0.012	0.144
	10	0.094	0.013	0.051	0.113	0.025	0.071	21	0.224	0.017	0.181
	20	0.117	0.018	0.074	0.145	0.038	0.103	24	0.279	0.023	0.236
Kiaga	Control	0.048	0.008	0	0.048	0.007	0	1	0.048	0.006	0
	5	0.051	0.012	0.003	0.060	0.018	0.011	17	0.251	0.009	0.203
	10	0.081	0.012	0.033	0.097	0.019	0.048	19	0.345	0.012	0.297
	20	0.124	0.016	0.076	0.157	0.029	0.109	27	0.441	0.039	0.393

[a]Effect = difference in Se concentration in grain between control and Se fertilizer treatments; [b]Increase = percentage increase in Se concentration for combining soil Se application with P and N; n = 4 for each treatment: each treatment was conducted in 4 experimental plots per study location

Table 19: The effect of Se fertilizer application techniques and doses on Se concentration (mg kg^{-1}) in bean grains on 3 locations in Kenya (Mbuyu, Mbeu and Kiaga)

		Soil Se application			**Soil Se application + N & P**				**Foliar Se application**		
Location	*Se (g ha^{-1})*	*Mean*	*SD*	*[1]Effect*	*Mean*	*SD*	*[1]Effect*	*[b]Increase*	*Mean*	*SD*	*[1]Effect*
Mbuyu	Control	0.018	0.004	0	0.018	0.003	0	1	0.018	0.003	0
	5	0.050	0.010	0.032	0.067	0.009	0.050	35	0.235	0.008	0.217
	10	0.086	0.006	0.068	0.094	0.012	0.076	9	0.482	0.011	0.464
	20	0.136	0.023	0.118	0.153	0.025	0.136	13	0.754	0.010	0.737
Mbeu	Control	0.022	0.006	0	0.023	0.004	0	1	0.023	0.005	0
	5	0.104	0.046	0.082	0.146	0.012	0.124	41	0.473	0.009	0.450
	10	0.171	0.031	0.148	0.204	0.019	0.181	19	0.873	0.038	0.851
	20	0.235	0.051	0.213	0.304	0.023	0.282	29	1.397	0.063	1.374
Kiaga	Control								0.048	0.006	0
	5								0.440	0.013	0.392
	10								0.873	0.042	0.825
	20								1.456	0.027	1.407

[a]Effect = difference in Se concentration in grain between control and Se fertilizer treatments; [b]Increase = percentage increase in Se concentration when combining soil Se application with P and N; N = 4 for each treatment: each treatment was conducted in 4 experimental plots per study location

5.3.4. Crop's response to Se fertilization

The study considered the ratio of Se concentration in grains of the 10 and 20 g ha^{-1} treatments relative to that of the lowest dose of 5 g ha^{-1} as reflecting the crop's response to Se fertilization (Table 20). In the soil Se fertilizer treatment (without P & N fertilizers), the response of maize to both 10 and 20 g Se ha^{-1} treatments was lowest in Mbuyu. However, when P and N fertilizers were combined with soil Se application, the response in maize was highest in Mbuyu for the 20 g Se ha^{-1} treatment. For 10 g Se ha^{-1} treatment, the response was highest in Kiaga followed by Mbuyu. For the foliar Se fertilizer treatment, the response to both 10 and 20 g Se ha^{-1} treatments in maize was highest in Mbuyu. Similarly in beans, the response was highest to all 10 and 20 g Se ha^{-1} treatments in Mbuyu and the foliar 20 g Se ha^{-1} treatment in Kiaga. Generally, the response to Se fertilizer was

highest at the study location with lowest Se concentration in control grains i.e. the high dietary Se deficiency risk zone.

Table 20: Grains' Se concentration increase ratio for the 10 and 20 g ha^{-1} Se fertilizer application doses relative to 5 g ha^{-1} for 3 fertilizer treatments on 3 crops (maize and beans) on 3 locations in Kenya (Mbuyu, Mbeu and Kiaga)

	Dose	**Maize**				**Beans**			
Location	*Se (g ha^{-1})*	*Soil*	*Soil + P&N*	*Foliar*	*Mean[a]*	*Soil*	*Soil + P&N*	*Foliar*	*Mean[a]*
Mbuyu	10	1.4	1.5	1.5	1.5	1.7	1.4	2.1	1.7
	20	1.8	2.9	6.1	3.6	2.7	2.3	3.2	2.7
Mbeu	10	1.8	1.4	1.2	1.5	1.6	1.4	1.8	1.6
	20	2.2	1.8	1.5	1.8	2.3	2.1	3.0	2.4
Kiaga	10	1.6	1.6	1.4	1.5	-[b]	-	2.0	2.0
	20	2.4	2.6	1.8	2.3	-	-	3.3	3.3
Mean[b]	10	1.6	1.5	1.3		1.7	1.4	2.0	
	20	2.2	2.4	3.1		2.5	2.2	3.2	

[a]Mean: mean ratio across study locations, [2]Mean: mean ratio per Se fertilizer application. -[b]: crop failure due to drought

Selenium concentration in grains increased linearly with increase in Se fertilizer application dose for both soil and foliar fertilizers (Table 21). Based on the linear regression's average R^2, all Se fertilizer application techniques and doses significantly contributed to explaining at least 80% of Se concentration increase in both maize and bean grains (p-value < 0.001), and the application of Se fertilizer was the main factor that increased Se concentration in grains. The regression slopes were higher in Mbeu and Kiaga compared to Mbuyu, meaning that the Se concentration increased in grains for the same fertilizer dose was lower in Mbuyu. In addition, these slopes for the soil fertilization of beans were of the same order of magnitude as those for the foliar fertilization of maize. This indicated that fertilizing beans more effectively resulted in an increase of Se concentration in the grains compared to fertilizing maize. Based on calculation of the ratio of regression slopes relative to the lowest slope of soil Se application in Mbuyu, foliar Se application had the highest impact in increasing Se concentration for both maize and bean grains.

Table 21: Results of linear regression between Se fertilizer application dose and Se concentration in grains for different fertilizer treatments (foliar and soil application) on 3 different locations in Kenya (p values < 0.001)

			Maize				**Beans**			
Fertilizer	*Application*	*Location*	*Slope[a]*	*Intercept[b]*	*R^2*	*Ratio[c]*	*[a]Slope*	*[2]Intercept*	*R^2*	*[c]Ratio*
Na_2SeO_4(aq)	Soil	Mbuyu	0.001	0.013	0.724		0.006	0.021	0.931	
		Mbeu	0.004	0.042	0.814	2.0	0.011	0.039	0.808	1.7
		Kiaga	0.004	0.041	0.856	2.0	-[d]	-	-	-
Na_2SeO_4(aq) Plus N & P	Soil	Mbuyu	0.003	0.009	0.932		0.007	0.026	0.923	
		Mbeu	0.005	0.052	0.733	2.5	0.014	0.049	0.925	2.2
		Kiaga	0.006	0.041	0.841	3.0	-	-	-	-
Na_2SeO_4(aq)	Foliar	Mbuyu	0.009	-0.011	0.894	4.5	0.037	0.005	0.979	5.7
		Mbeu	0.011	0.089	0.802	5.5	0.068	0.095	0.769	10.5
		Kiaga	0.018	0.110	0.866	9.0	0.070	0.088	0.989	10.8

[a]Slope: mg kg^{-1}/g ha^{-1}, [b]Intercept: g Se ha^{-1}, [c]Ratio: regression slopes ratios relative to the lowest slope of soil application in Mbuyu. [d]: crop failure due to drought

5.3.5. Effect of full factorial combination of Se, Zn, and I fertilizers on Se concentration of grains

The study tested the crop's response to Se fertilization when simultaneously applied with Zn and I fertilizers, i.e. whether the relationship between Se fertilization and Se concentration in grains varied across the levels of either Zn or I fertilizers or both. At all study locations, there was no significant 3-way interaction effect for the Se, Zn, and I fertilizer combinations on Se concentration in maize grains. Selenium fertilizer application was the main variable with a significant large effect on Se concentration in maize grains (p-value < 0.001), with an effect size index of 0.219. Moreover, there was no significant 2-way interaction effect for either Se and Zn or Se and I, on Se concentration in both maize and bean grains. Similarly, Se fertilizer application remained the main variable with a significant large effect on Se concentration in grains (p-value < 0.001); effect size index = 0.379, 0.852, and 0.402 for the 2-way combinations of Se and Zn on maize, Se and Zn on beans, and Se and I on maize, respectively. The assumption for homogeneity of variance was not met for the Se, Zn, and I fertilizer combinations for beans. Figure 7 illustrates the mean Se concentration in maize grains for all possible full factorial combinations of Se, Zn, and I fertilizer. The large effects of the 5, 10, and 20 g ha^{-1} Se fertilizer treatments can clearly be noted, while the plateau sections of the graph represent the insignificant effects of the additional Zn and I fertilizer treatments.

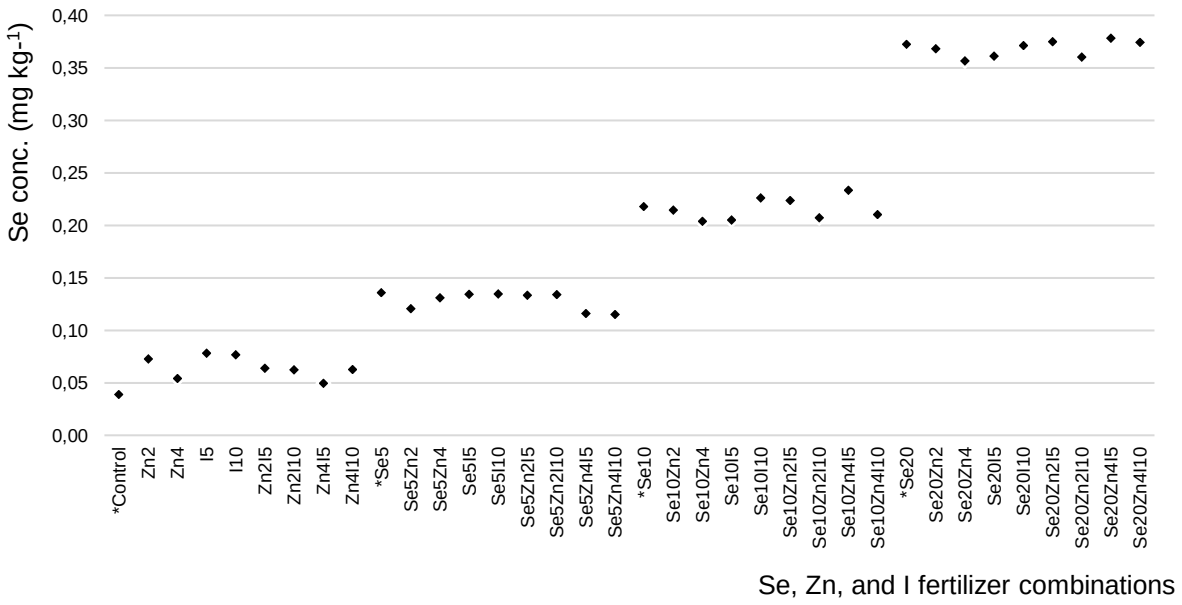

Figure 7: Mean Se concentration in maize grains (mg kg^{-1}) for all the possible foliar full factorial combinations of Se, Zn, and I fertilizer doses: 4*Se (0, 5, 10, 20) g ha^{-1}, 3*Zn (0, 2, and 4) kg ha^{-1}, and 3*I (0, 5, 10) g ha^{-1}

5.4. Discussion

Across the three study locations, Se concentration in maize and bean grains for the control treatment was within the Se range previously reported for cereals that was considered as low, i.e. 0.005 to 0.02 mg Se kg^{-1} 1 (Dhillon and Dhillon 1999). The moderately acidic soils at the three study locations restricts Se availability for plants' uptake, resulting in the low Se concentration in grains. Selenite, which is less water soluble is the predominant Se form in acidic soils (Gupta and Gupta 2017). The low Se concentration in the grains relates to median maize grain Se concentration reported in Malawi at 0.019 mg kg^{-1}, in regions characterized with widespread sub-optimal dietary Se intake (Chilimba et al. 2011). This also compares to concentrations of 0.0032 mg kg^{-1} reported in corn from Se deficient regions of Northeast China, 0.005 to 0.12 mg kg^{-1} in cereal products for pre-1984 period in Finland, or 0.004 to 0.09 mg kg^{-1} in New Zealand (Combs 2001).

From this study, soil and foliar applications of sodium selenate fertilizer significantly increased the Se concentration in both maize and bean grains in the Central Kenya Highlands. Selenate is the most bioavailable form of Se, and therefore readily taken up by plants via roots or foliar pathways (Gupta and Gupta 2017). On average, soil Se application (without P and N fertilizers) resulted in Se concentrations in maize grains of 0.042, 0.068, and 0.093 mg kg^{-1} for the 5, 10, and 20 g Se ha^{-1} fertilizer applications, respectively. Foliar application of the same doses resulted in maize grain concentrations of 0.157, 0.205, and 0.306 mg kg^{-1}, respectively. On average, the Se concentration in maize grains increases by 18 µg kg^{-1} for each g ha^{-1} of Se applied as sodium selenate. This compares to a Se concentration increase in maize grains of 20 µg kg^{-1} in Malawi (Chilimba et al. 2012). A foliar Se fertilizer dose of 11 g Se ha^{-1} applied by Wang et al. (2013) resulted in a Se concentration of 0.096 mg kg^{-1} in maize grains, which is lower than the concentration we have observed for the 10 g Se ha^{-1} treatment. However, studies conducted using other cereals reported comparable results as those of our study. For instance, foliar Se application at 25 g Se ha^{-1} on upland rice increased the Se concentration in the grains to 0.320 mg kg^{-1} (Reis et al. 2018), which is similar to the concentrations we found for maize in our 20 g Se ha^{-1} foliar treatment. Wang et al. (2017) also reported a Se concentration of 0.310 mg kg^{-1} in wheat, after foliar Se application at a dose of 30 g Se ha^{-1}. Lyons et al. (2004b) reported Se concentrations of 0.150 and 0.350 mg kg^{-1} for 4 and 12 g Se ha^{-1} foliar treatments, respectively, which are comparable to concentrations we found in maize for the 5 and 10 g Se ha^{-1} foliar treatments. However, their soil Se application resulted in a Se concentration of 0.210 and 0.360 mg kg^{-1}, respectively, which is much higher compared to our findings. This can be explained by the relatively higher soil pH (H_2O) of 6.6 in their study. Also Ducsay et al. (2016) and Chen et al. (2002) reported results in line with our findings. Ducsay et al. (2016) found an increase of Se concentration in winter wheat to 0.445 mg kg^{-1} for 10 g Se ha^{-1} foliar application, and Chen et al. (2002) reported a Se concentration increase in rice to 0.640 mg kg^{-1} for 20 g Se ha^{-1} foliar application.

As the study was conducted with different soil types, variation in geochemical interactions with Se upon soil fertilization influenced the magnitude of effect on Se concentration in grains across the study locations. Compared to Mbeu and Kiaga, Se concentration increase in maize and bean grains for the same fertilizer dose and technique was lower in Mbuyu. This can be explained by the lower soil Fe, Al, and P concentration, which are the main soil characteristics that differ from Mbeu and Kiaga. In the soil, Se is adsorbed by Fe/Al oxides (Blaylock and James 1994), so lower Fe/Al oxides is expected to result in less Se adsorption. Notably, soil P forms insoluble Fe and Al phosphates that are more strongly adsorbed in the soil than Se and hence, adsorption of phosphates reduces Se adsorption (Dhillon and Dhillon 2000, Eich-Greatorex et al. 2010). However, the lower soil P in Mbuyu indicates less competition for Se to adsorb onto the soil, resulting in low Se concentration in the grains. Using the P to Fe ratio as a parameter predicting the potential P availability in the soil, Mbuyu has the lowest P to Fe ratio of 6.5, compared to 16 for Mbeu and 25 for Kiaga. The higher P to Fe ratio in Mbeu and Kiaga suggests higher saturation of sorption sites by P at these locations and hence higher Se availability/mobility in soil. This may also explain the significant effect of P and N fertilizer on Se concentration in maize grains for soil Se fertilizer application at 20 g ha^{-1} in Mbuyu. By adding P fertilizer, Se mobility and availability was mainly affected in Mbuyu. Retention of Se in the soil decreases with increase in PO_4^{3-} and consequently, Se remains in the soil solution for plant uptake (Dhillon and Dhillon 2000). Conversely, an increase in PO_4^{3-} content induces plant growth and hence root development that increase Se uptake by crops. However, this increase in Se content is diluted by the increased biomass. This explains the lack of significant effect of addition of PO_4^{3-} on Se concentration in grains in Mbeu and Kiaga with relatively higher soil P content. In such locations, addition of PO_4^{3-} has no impact on soil Se availability as all the sorption sites are already saturated with P and hence, the added PO_4^{3-} therefore mostly supports crops' growth. However in Mbuyu, the location with the lowest soil P content, the margin to saturate the sorption sites with PO_4^{3-} is wider. As a consequence, most of the PO_4^{3-} added displaces Se from fixation and less of it left to support crop's growth. As a consequence, the increase in Se uptake from both the desorbed and applied Se supersedes any neutralization by increased biomass. This explains the positive effect of combining soil Se fertilizer application with P and N fertilizers on Se concentration in the grains in Mbuyu. If this would have been a negative effect, the effect could have been due to dilution as a result of higher crop yield. However, this is not the case.

Moreover, soil organic matter which is an important property in tropical soil fertility (Lal 2006), is also lower in Mbuyu, which may have affected Se availability in the soil. Low organic matter in Mbuyu is a result of lower rainfall and air/soil temperature, which are primary factors influencing soil organic matter in the region. Crop residue inputs that are needed to maintain soil organic matter are variable depending on the climate and management, of which in arid areas such as Mbuyu, climate has more weight. In terms of soil management, continuous tillage decreases soil organic matter, which is the case in the restricted parcels of land that these rural farmers depend on for food production, leaving no room for the traditional rotational farming (Giachene and Kimaru 2003, Karmakar et al. 2016).

In this study, foliar Se fertilizer application significantly increased Se concentrations in maize and beans, more than soil fertilizer application did. These findings are in line with the fact that foliar pathways are commonly recognized as potentially more effective for Se uptake by plants because Se immobilization in the soil is avoided (De Temmerman et al. 2014, Lawson et al. 2015). In our study, the increase in Se concentration in grain was significantly higher in beans than in maize. This can be attributed to differences in plant species (Zhao et al. 2005), with the higher Se concentration in beans being attributed to higher protein content. Selenium incorporation into protein amino-acids is the dominant feature in Se non-accumulator plants (Peterson and Butler 1962). Crops moderately accumulate Se in their edible parts, with Se levels varying among crop species in the order: brassica > bean > cereals (Gupta and Gupta 2017). Plants in the Fabaceae family therefore incorporate more Se than plants in the Gramineae family (Vineyard 2010). In our study, a comparison of the regression slopes between Se fertilizer application dose and Se concentration in maize and bean grains shows that the effect of the soil Se application on Se concentration in bean grains is of the same order of magnitude as the effect of foliar application on Se concentration in maize grains. Considering that soil fertilization has less effect on Se concentration in grains, only fertilizing beans may be more efficient in improving dietary Se supply than maize.

In this study, a comparison of crops' response to Se fertilization based on the ratio of Se concentration in grains for the 10 and 20 g ha^{-1} treatments relative to that of the lowest dose of 5 g ha^{-1} indicates that the crops' response to Se fertilization is actually highest in Mbuyu. This implies that Se fertilization potentially is more efficient in increasing Se supply at locations at higher risk of dietary Se deficiency. Based on dietary intake data from Chapter 3, it was projected that a grain Se concentration of 0.3 mg kg^{-1} would improve average dietary Se intake to adequate intake levels. However, this biofortification target Se level was only achieved in Mbeu and Kiaga with a foliar Se application dose of 20 g ha^{-1} on maize, which corresponds to a grain Se concentration increase of 11.8 and 19.7 µg kg^{-1} for 1.0 g Se ha^{-1} foliar application, respectively. By extrapolating the results from Mbuyu, the biofortification target Se level of 0.3 mg kg^{-1} would be achieved by a foliar Se application dose of 31 g ha^{-1} on maize, corresponding to a grain Se concentration increase of 10.4 µg kg^{-1} for 1.0 g Se ha^{-1} foliar application. This is in line with a Se application dose of 34 g ha^{-1} on maize, required to achieve the biofortification target level for Se in a Se deficient region of China (Mao et al. 2014). In the case of beans, a Se fertilizer application dose of 10 g ha^{-1} achieves the biofortification target Se level in Mbuyu, which corresponds to a Se concentration increase of 46.4 µg kg^{-1} for 1.0 g Se ha^{-1} foliar treatment. A lower Se application dose of 5 g ha^{-1} is however required in Mbeu and Kiaga, that corresponds to an average Se concentration increase of 84.2 µg kg^{-1} for 1.0 g Se ha^{-1} foliar application.

In our study, fortifying maize and beans crops with a combination of Se, Zn, and I fertilizers was found not to result in a significant interaction effect on Se concentration in maize grains. Selenium fertilizer application dose remains the main variable with a significant large effect on Se

concentration in grains, even after addition of Zn or I fertilizer at different doses and combinations. In a Se deficient region of China, also no significant difference was observed in Se concentration between Se-only treated and Se+Zn+1 treated maize, soybean, potato, and cabbage. In addition, the Zn or I concentration in these foods after respective Zn and I treatments was statistically identical to the Zn or I concentration after the Se+Zn+I treatment (Mao et al. 2014). This confirms that a multi-mineral agronomic biofortification increases Se, Zn, and I concentrations in staple grains, and potentially can be implemented to address the three mineral deficiencies simultaneously.

Based on the estimated average daily dietary Se intake data for under-5 children and women from 19 to 39 years old in Mbuyu, a foliar Se application of 10 g ha^{-1} on both maize and bean crops would improve daily Se intake from 7.6 and 14.4 µg day^{-1} to adequate intakes levels of 27 and 54 µg day^{-1}, for children and women, respectively. The estimated average requirements for Se are 17 µg day^{-1} for children 1 - 3 years, 23 µg day^{-1} for children 4 - 8 years, 35 µg day^{-1} for children 9 -13 years, and 45 µg day^{-1} for children >14 years and adults (Institute of Medicine 2000). Foliar application of 10 g Se ha^{-1} corresponds to a grain Se concentration increase of 3.6 µg kg^{-1} in maize and 46.4 µg kg^{-1} in beans per 1.0 g Se ha^{-1} applied. Since our study found that beans respond better to Se fertilizer than maize, a case scenario where only bean crops are biofortified with a foliar application of 10 g Se ha^{-1}, would result into an adequate dietary Se intake of 25 and 51 µg day^{-1} for the children and women, respectively. Therefore, biofortification of beans alone also achieves estimated daily requirements. However, if only maize crops are biofortified, even a foliar application of 20 g Se ha^{-1} would result in an inadequate dietary Se intake of 16 and 33 µg day^{-1} respectively. The extrapolated Se application dose of 31 g ha^{-1} on maize for Mbuyu, would instead be suitable for locations at high risk of dietary Se deficiency, as was recommended by Mao et al. (2014). Generally, the bean crops are better target crops for agronomic Se biofortification, while maize crops would require a foliar Se fertilizer application at a dose >20 g Se ha^{-1} in more Se deficient locations. Our study contributes to validation of current recommendation systems for Se agronomic biofortification in arable systems. The results point toward the potential of a multi-mineral agronomic biofortification strategy to address multiple mineral deficiencies, based on a site-specific biofortification strategy.

It should however be noted that both soil Se fertilizer application and overflows of foliar Se fertilizer application may result in Se retention in the soil which in the long-term may result in Se accumulation in the soil. Selenium biofortification intervention at a national or regional level would therefore require continuous monitoring and evaluation of the Se status in the environment, and especially in the water bodies where Se from run-offs may eventually accumulate. In the ecosystem, Se is toxic to aquatic life in relatively low concentrations and has been linked to adverse ecological effects in aquatic ecosystems such as reproductive and developmental impairment of aquatic birds and fish (Skorupa 1998, Sappington 2002).

CHAPTER 6: IMPROVING DIETARY SELENUM INTAKE THROUGH AGRONOMIC BIOFORTIFICATION OF MAIZE CROPS IN KENYA

6.1. Introduction

While the range of adequate Se intake is relatively narrow as compared to other minerals, inadequate Se intake results in in clinical disorders or act as an exacerbating factor in infectious diseases (White and Broadley 2005). This deficiency has mainly been attributed to low soil Se phytoavailability, resulting in intake of foods with low Se concentration. This is further aggravated by diets subsisting on cereals and limited consumption of sea food or animal products (White and Broadley 2008, Welch and Graham 2005). Developing countries suffer the most from such "silent epidemics", affecting mainly children and women of childbearing age (Darnton-Hill et al. 2005). Yet, Se deficiency has not received the same research and intervention attention as deficiencies in Fe, Zn, or I.

Existing measures to address mineral deficiencies include dietary diversification, commercial fortification of processed foods, and direct supplementation. While supplementation enables to reach portions of the population most at risk, it has long-term challenges related to sustainability, coverage, and compliance of the affected population groups. Fortification of widely used basic processed foods on the other hand demands that the food vehicle be universally purchased or distributed. The coverage and its effectiveness are often suboptimal in developing countries as the affected rural population depends primarily on subsistence agriculture. Thus, in the context of a rural population in a developing country, population-based strategies are the most feasible and effective way to intervene on communities' mineral deficiencies and meet their micronutrients' needs (Harrison 2010).

Plant-based biofortification is the most commonly used population-based strategy for enriching staple crops with specific minerals, and is increasingly being adopted in developing countries, i.e. in contexts where commercial fortified food is not feasible (White and Broadley 2009, Tanumihardjo 2008, Bouis and Welch 2010). This strategy aims to improve the nutritional trace elements lacking in the human diet through intervening on crops and therefore on readily available foods (Welch 2001, Masset et al. 2012). Cropping systems are thus modified to meet human nutritional needs. Depending on the targeted mineral, biofortification may be realised in different ways. For instance, in regions where soils are deficient in minerals essential for both the human body and plant growth, such as Zn, farmers can use micronutrient fertilizers to simultaneously improve the crop's yields and the nutritional value of their food (Graham et al. 2007). In fact, most of the agronomic biofortification programs targeting staple foods use Zn (Bouis et al. 2011). However, Se is only essential for humans and animals but not for plants. Accordingly, supplementation of Se does not lead to improved crop growth and farmers and fertilizer producers have not yet been stimulated to supply Se to the crops so far.

Different forms of Se may be used for Se biofortification, resulting in different amounts and chemical forms of Se accumulated in plants (Schiavon et al. 2013). Selenate is usually preferred

over other forms like selenite or selenomethionine in the case of foliar application, as it is more readily absorbed and accumulated by plant's leaves (Kikkert and Berkelaar 2013). Moreover, Se biofortification is more successful for crops that accumulate Se in edible parts, such as in cereal grains, which therefore can serve as key "Se-delivery vehicle" for diets in Se deficient regions. Cereal based foods are the major source of minerals, with most countries having a single cereal as the primary staple. In developing countries, the most widely consumed cereals are rice, wheat, maize, sorghum, and millet (Serna-Saldivar 2010). Maize is the most important food staple in the East and Southern African region (Tefera 2012) where per capita maize consumption is higher than average (Pingali 2001). In such contexts, maize is therefore a relevant candidate for Se agronomic biofortification intervention.

Previous studies on Se biofortification interventions applied selenate as the inorganic Se fertilizer, or it was supplemented in ordinary fertilizers, for the cultivation of food crops (Fordyce 2005). Most of them, whether in developed or in developing countries, showed a successful effect of the fertilization on crops. In Finland for instance, sodium selenate fertilizers have been applied to agricultural soils since 1985 and have successfully increased Se concentration in many foodstuffs by over 10-fold (Ekholm et al. 2007). In Canada, to protect against Se deficiency, soil and foliar selenate application or seed treatment at 10 g Se ha^{-1} resulted in sufficiently Se enriched crops (Gupta and Gupta 2002). In the UK, application of 10 g ha^{-1} of selenate increased total Se concentration in wheat grain by 10-folds (Broadley et al. 2010), and by 6.4 and 7.1 µg Se per average slice of white and whole bread respectively (Hart et al. 2011). In China, selenate foliar application of 20 g Se ha^{-1} increased Se concentration in rice grains by 9.0 folds (Chen et al. 2002). In Africa, foliar selenate application increased the Se concentration in maize grains by 0.020 mg kg^{-1}, for each gram of Se applied, and hence, a Se fertilizer application rate of 5 g Se ha^{-1} on maize crops was estimated to increase dietary Se intake by 26 - 37 µg d^{-1}, based on national maize consumption patterns in Malawi (Chilimba et al. 2012). Current recommendation systems for Se agronomic biofortification have established a Se soil/foliar application dose of approximately 10 g Se ha^{-1} for arable systems. In most cases, the response of crops is linear with the Se dose applied (Ros et al. 2014). However, it should be noted that Se is not only an essential nutrient for humans, but it is also an environmental toxicant; the boundary between the two roles is narrow and depends on its chemical form, concentration, and other environmentally influenced variables. Trace concentrations of Se are required for normal growth and development, moderate concentrations can be stored to maintain homeostatic functions, but elevated concentrations can result in toxic effects (Hamilton 2004, Rayman 2008).

The ultimate goal of a Se biofortification intervention is to increase dietary Se intake to a level that meets body requirements. In the past, Se intakes of individuals or populations were assessed based on standard FCTs and per capital food consumption or food frequency questionnaire data. A limitation of this approach is that FCTs do not capture the geographical variation in food Se concentration, leading to biased estimates of Se intakes. Randomized control trials have also been

conducted to examine the impact of a Se biofortified food, using plasma Se concentration as the biomarker of Se status (see Hurst et al. 2010, Khan et al. 2017). However, in resource limited settings such as developing countries, the sampling, transport, and analysis of biological Se status biomarkers such as serum Se, is not always feasible. No study was found that evaluated the impact of Se biofortification by measuring average dietary Se intake by the population after the biofortification intervention. Analysing the actual Se concentration of a representative sample of consumed foods, and assessing the estimated dietary intake of the affected population, allows a valid estimation of average dietary Se intake of individuals or populations, which is calculated as the sum of the products of the Se concentration in different food products and their consumed amounts (Combs 2015). Since food Se is generally well utilized, with selenate having over 84% absorption rate into the body (Van Dael et al. 2002), the study aimed at studying the relation between crop biofortification and dietary intake. This would enable evaluating whether biofortification can help determine whether populations meet the recommended Se intakes (Wolfram et al. 1985, Turner et al. 1990).

This study investigated the effect of foliar Se fertilization on the Se concentration in maize grains and its impact on the average daily dietary Se intake at a particular location in the central Kenya highlands previously identified to be at risk of dietary Se deficiency. By doing so, the study contributes to the evaluation of the effectiveness of agronomic biofortification strategies in increasing dietary Se intake and therefore reducing the risk of Se deficiency in the human population. The study hypothesized that Se biofortification intervention on maize would decrease the risk of dietary Se deficiency. This is mainly because maize consists of a large portion of the population's diet and hence, will have a positive impact on the dietary Se intake of the target population. The study contributes to the understanding of possible interventions that could be used to reduce the risk of dietary Se deficiency in a population, necessary for the development of well-targeted policies. Finally, the study enables a better understanding of factors affecting the Se concentration in local foodstuffs and hence, the risk of dietary Se deficiency.

6.2. Materials

6.2.1. Location

The study was conducted in Kiaga region, located in the central Kenya highlands, on the Southern foot of Mt. Kenya. Kiaga has an elevation of 1,207 meters (0.62^{o}S 37.25^{o}E) and experiences a tropical savanna climate. Verto-eutric nitisols are the main agricultural soils, formed from Mt. Kenya's volcanic rocks. The annual temperature is on average 21.6 ^{o}C, with March being the warmest month at 23 ^{o}C and July the coolest at 19.9 ^{o}C. The region has two distinct rainy seasons in April and November and a dry weather throughout the year, with an average annual rainfall of 1,149 mm (Dijkshoorn et al. 2011). Based on an analysis of soil and food Se concentration data in

the Se status survey (Chapter 3), Kiaga is characterized by Se deficient agricultural soils, low Se concentration in locally grown foodstuffs, and sub-optimal dietary Se intake among the local population. Total soil Se concentration varies from 0.284 to 0.653 mg kg^{-1}, with a median of 0.357 mg kg^{-1}, which is below the soil Se deficiency threshold of 0.6 mg kg^{-1} in agricultural soils (Fordyce 2013). Low Se concentrations in local foodstuffs result in inadequate median dietary Se intakes, equal to 16 µg day^{-1} among under-5 children and 27 µg day^{-1} among women. Maize is the most commonly consumed staple cereal grain and is locally grown in Kiaga. According to estimated dietary intake assessment data from the previous Se status survey on the study location, over 70% of under-5 children and women were reported to have consumed either maize grains or maize-based meal, which constitute a dominant portion of the daily local standard diet. The Se agronomic biofortification intervention trial in this study was therefore conducted on maize.

6.2.2. Sample population and randomization

The study targeted children aged 6 to 59 months and women aged 19 to 39 years, due to their vulnerability to micronutrient deficiencies (Darnton-Hill et al. 2005). A village was used as the unit of randomization as it is the lowest administrative unit in Kenya and geographically identified with an intact community. Required sample size was calculated for unmatched cluster-randomized trials according to Hayes and Bennett (1999), with the proportion of the target population with inadequate Se intake (at risk of dietary Se deficiency) as the primary outcome measure. Number of participants (n) to be sampled for each group was calculated using the formula:

$$n = (z_{\alpha/2} + z_{\beta})^2[\pi_0 (1- \pi_0) + \pi_1 (1- \pi_1)]/ (\pi_0 - \pi_1)^2 = 30$$

Then c, the number of clusters required is given by:

$$c = 1 + (z_{\alpha/2} + z_{\beta})^2[\pi_0 (1- \pi_0)/n + \pi_1 (1- \pi_1)/n + k^2 (\pi_0^2 + \pi_1^2)]/ (\pi 0 - \pi_1)^2 = 4.8 \quad (5)$$

Where $z_{\alpha/2}$ is the statistical significance of 5% ($p < 0.05 = 1.96$, two-tailed test), z_{β} is the power of 80% ($z_{\beta} = 0.80$), π_1 and π_0 are the true (population) proportions in the presence (estimated 20% Chapter 5) and absence (52% Joy et al. 2014) of the intervention respectively, and k is coefficient of variation of true proportions between clusters which is often ≤ 0.25, and seldom exceeds 0.5 for most health outcomes i.e. the average 0.375 was used (Hayes 1999). From the formula, 5 clusters were needed per treatment group to detect at least 32% difference in Se deficiency risk between intervention and control groups. A total of 30 households per treatment group were enrolled in each treatment group. Considering possible power reduction resulting from losses due to refusals to participate or migration, the initial sample size was increased by 10% i.e. 33 households per treatment group. However due to drought in the study location at the time of village allocation to the treatment arms, four of the ten villages in Kiaga were exempted from the study. Only the remaining six villages were randomly allocated to the trial arms. As a result, three villages were each allocated at random to the intervention and control groups. As a result, the power to detect

the effect size based on 5 villages per trial arm was reduced by the presence of drought in the study location. In this case, 13 households were randomly selected from each village.

According to regional climate classifications, the six villages within Kiaga have similar climatic and agricultural soil type (Jaetzold et al. 2006). Randomization of villages to the treatment groups was achieved by obtaining computer generated random numbers. The intervention group consisted of the farms that were the object of Se biofortification on maize crops, while the farms of the control group received a placebo (none of the crops were biofortified). Due to lack of local residents register data, households with under-5 year old children from each village were identified by means of a systematic door-to-door survey and assigned an identification number. Using a random number table, 7 households were selected for each village. All of these (and only these) households participated in the study. In case a household declined to participate, it was replaced by the next household on the list. Excluded from participation were women and children with congenital or chronic abnormalities impairing feeding pattern, severely ill, with clinical conditions under feeding regimes deviating from a regular diet, or non-permanent residents of the study location.

6.3. Method

6.3.1. Study design and outcome measures

The risk of dietary Se deficiency of children and women was the primary outcome of this study. The secondary outcome measures are the change in Se concentration in maize grains, the dietary Se intake from maize, and its impact on the daily average dietary Se intake. Data collection (i.e. foodstuff sampling and dietary intake assessment) was conducted during the planting season in April 2017 (baseline) and in August 2017 (post-trial) for both the intervention and the control groups. The baseline data collection was done after all subjects were informed about the study objectives, procedures and measurement methods, and signed a consent form. A consort flowchart (Figure 8) summarizes the recruitment, allocation, follow-up and analysis of the effect of Se biofortification of maize on the risk of dietary Se deficiency in Kiaga. The study was approved and cleared by Meru University of Science & Technology Institutional Research Ethics Review Committee (MIRERC) in Kenya (MIRERC/016/2017), and was registered in the Pan African Clinical Trials Registry (PACTR) with trial number PACTR201710002698231.

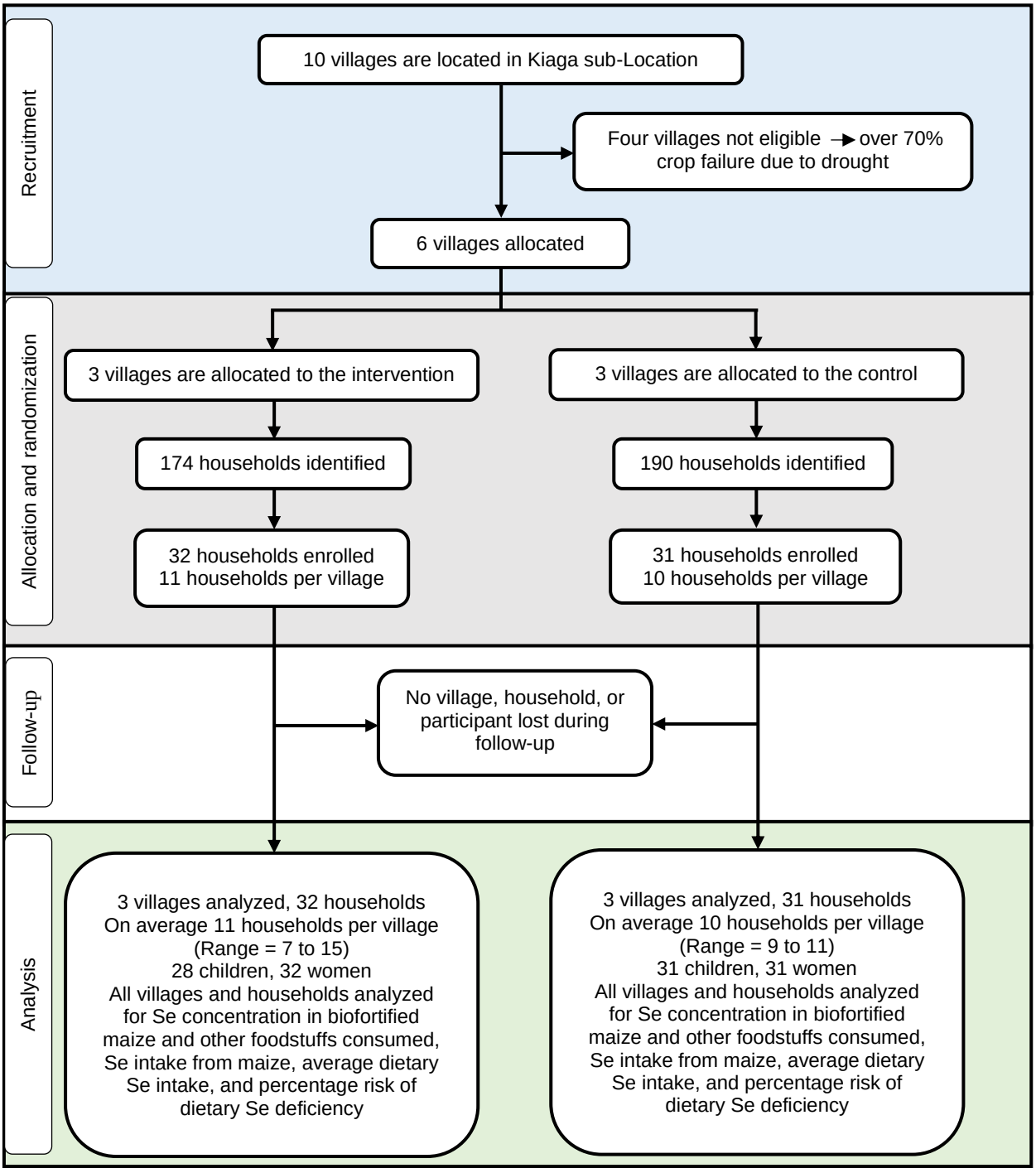

Figure 8: Recruitment, allocation, follow-up and analysis of the effect of Se biofortification of maize on the risk of dietary Se deficiency in Kenya

6.3.2. Biofortification procedure

The results from a Se biofortification trial on maize crops in Kiaga - Chapter 5 - showed that foliar application resulted in a Se concentration in maize grains that was four times higher than in the case of soil fertiliser application. Thus, this study biofortified maize crops using foliar Se application. For the treatment group, a Se fertilization dosage of 20 g Se ha^{-1} in form of sodium selenate

(Na_2SeO_4) was applied on maize crops. This dosage was selected as it was projected to improve the daily average dietary Se intake by 55%, and decrease estimated risk of dietary Se deficiency by 32% in Se deficient zones of the central Kenya highlands (Chapter 5). Following the local cropping calendar, on average 1 acre of farm per household participating in the study was ploughed and harrowed in February 2017. Sowing holes of 3 cm deep were prepared in each farm, with the spacing between rows being 75 cm and the spacing between holes being 30 cm. After the early rains in April, two maize seeds of the variety KH 500-33A, were sowed in each hole. When the crops were at the stem elongation stage, Se fertilizer was evenly sprayed on crops' leaves with the applicants wearing protective clothing. The final Se concentration was 0.062 g L^{-1} and was sprayed in 320 L of water per acre of maize crops. For the control group, nothing was applied on the maize crops.

6.3.3. Food sampling and dietary intake assessment

At both baseline and post-trial, dietary intake assessment was conducted on the same children and women (mothers/guardian on behalf of the children) as described in section 2.2.1. Average dietary Se intake (µg d^{-1}) was assessed by multiplying the daily amount of biofortified maize and other non-biofortified foodstuffs consumed by their respective Se concentration. Average dietary Se intake was compared to EARs to estimate the percentage change in risk of dietary Se deficiency. The difference-in-differences approach was therefore used to estimate the effect of the intervention on the two outcomes of the study.

Food sampling was conducted at baseline and at post-trial when the maize crops reached physiological maturity. While only maize crops were Se biofortified in the treatment group, sampling and analysis of actual Se concentration in all other foods consumed by the study population was conducted to estimate the average daily dietary Se intake as described in sections 2.2.2 and 2.3, respectively. Foodstuffs sampled from households included cereals, legumes, animal-source foods, vegetables, and fruits. Additionally, the study measured nutritional status of the study participants and soil geochemical characteristics for the control and the treatment groups at baseline as described in section 2.2.1 and 2.3, respectively.

6.3.4. Analysis of the primary and secondary outcomes

Statistical analysis was performed with SPSS - IBM Corp. (2016). IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY. Prior to analysis, outliers were identified by Tukey's Hinges inter-quartile range rule. Outliers above the 3 SD or below -3 SD were excluded. Descriptive statistics analysis for Se concentration in foodstuffs, soil geochemical characteristics, participants' nutritional status, and dietary Se intake data were based on untransformed data. Normal distribution of data was verified by Shapiro-Wilk Test. Difference between means was tested using

Independent-Samples T-Test. Assumptions for linearity and homogeneity of variance were met. To account for the clustered nature of the data, difference-in-difference method based on a two-level mixed model for clustered data was used to determine the effect of Se biofortification intervention on Se concentration in maize grains. The model was also applied to test the effect of Se biofortification on Se intake from maize grains, and on the average daily dietary Se intake. A confidence level of 95% was applied.

6.4. Results

6.4.1. Trial groups comparison at baseline

The intervention trial did not have any dropout of villages nor participants between the baseline and post-trial phases as was illustrated in the consort flowchart in *Figure 8*. The nutritional status and dietary intake data of the study population and soil geochemical characteristics of the trial farms at baseline are presented in Table 22 below. Considering the overall study population i.e. control and treatment groups combined, 59% were girls and 41% were boys among the children, with a mean age of 3 years (SD = 1.3). Anthropometric indices based on age, weight, and height measurements show that 8.5% of the children were stunted, 3.4% underweight, and 3.4% wasted, as indicated by z-scores for height-for-age (HAZ), weight-for-age (WAZ), and weight-for-height (WHZ) indices, respectively. The mean age among women was 31 years (SD = 7.5), with a mean body mass index (BMI) of 24.8 (SD = 5.1). On average, 46.8% of the women were overweight and 12.9% were obese. There was no significant difference between the mean energy intake of 866 kcal (SD = 368) in the control group and 1087 kcal (SD = 610) in the treatment group for children (p-value = 0.087), and between 2067 kcal (SD = 658) in the control group and 2112 kcal (SD = 791) in the treatment group for women (p-value = 0.805). For the soil geochemical characteristics, the mean total soil Se concentration was equal to 0.316 in the control farms and 0.333 mg kg^{-1} in the treatment farms; this was not significantly different (p-value = 0.074). Considering the KH_2PO_4-extractable soil Se concentration, both control and treatment farms had a mean value equal to 0.006 mg kg^{-1} (p-value = 0.35). The other soil geochemical characteristics are shown in Table 22.

Table 22: Baseline anthropometric data, dietary Se and energy intake, dietary diversity scores and number of animal-source foods consumed in 24 hours, and soil geochemical characteristics in Kiaga for both the control and treatment group

		Control		Treatment	
	Baseline characteristics	Mean	SD	Mean	SD
Children	Age (years)	3.0	*1.3*	2.5	*1.3*
	Weight (kg)	12.1	*2.9*	13.3	*3.7*
	Height (cm)	91.3	*11.4*	87.6	*12.1*
	WAZ	-0.6	*1.5*	-0.4	*1.0*
	HAZ	-0.2	*2.5*	0.0	*1.7*
	WHZ	-0.2	*0.9*	-0.6	*0.9*
	Energy (kcal)	866	*368*	1087	*611*
	Dietary diversity scores (DDS)	4.3	*1.1*	4.1	*1.2*
	Animal source food	1.3	*0.4*	1.2	*0.4*
	Average dietary Se intake (µg d^{-1})	10.50	*5.03*	10.92	*6.94*
Women	Age (years)	31.2	*7.5*	29.9	*6.7*
	Weight (kg)	61.4	*13.8*	60.5	*10.1*
	Height (cm)	157.2	*5.0*	155.5	*5.7*
	Body Mass Index (kg m^{-2})	24.8	*5.1*	25.0	*3.9*
	Energy (kcal)	2067	*658*	2112	*791*
	Dietary diversity scores (DDS)	4.7	*1.0*	4.5	*1.0*
	Animal source food (*counts)	1.3	*0.6*	1.3	*0.6*
	Average dietary Se intake (µg d^{-1})	21.95	*11.61*	19.31	*10.43*
Geochemical characteristics	Total Se (mg kg^{-1})	0.316	*0.044*	0.333	*0.037*
	Extractable Se (mg kg^{-1})	0.006	*0.003*	0.006	*0.002*
	Organic matter (% OM)	0.216	*0.029*	0.204	*0.038*
	Organic carbon (% OC)	0.125	*0.017*	0.119	*0.022*
	$CaCO_3$ (%)	0.050	*0.006*	0.049	*0.007*
	pH-H_2O	6.2	*0.3*	6.2	*0.3*
	pH-KCl	5.1	*0.3*	5.0	*0.3*
	Mn (g/kg)	2.9	*1.3*	2.8	*1.3*
	Cu (mg kg^{-1})	34	*9*	34	*9*
	Ni (mg kg^{-1})	33	*18*	34	*18*
	Co (mg kg^{-1})	41	*12*	41	*11*
	Cr (mg kg^{-1})	44	*25*	46	*24*
	Fe (g/kg)	124	*7*	122	*10*
	Al (g/kg)	102	*13*	101	*13*
	Zn (mg kg^{-1})	128	*27*	126	*24*
	S (mg kg^{-1})	271	*90*	282	*91*
	Na (mg kg^{-1})	161	*94*	167	*99*
	K (mg kg^{-1})	1108	*407*	1083	*409*
	Ca (mg kg^{-1})	2695	*1088*	2698	*1052*
	Mg (mg kg^{-1})	2598	*682*	2555	*657*
	P (mg kg^{-1})	2679	*1381*	2564	*1307*
	N (mg kg^{-1})	2309	*1068*	2408	*1071*

*counts: number animal-source foods consumed in 24 hours

6.4.2. Differences and mean changes in Se concentration in maize grains, Se intake from maize, and average daily dietary Se intake after intervention

Selenium fertilization at a dosage of 20 g ha^{-1} significantly increased Se concentration in maize grains on average from a control level of 0.019 to 0.118 mg kg^{-1}, which corresponds to on average 5 µg kg^{-1} increase in Se concentration in maize grains for each gram of Se applied as sodium selenate. Compared to the control group, the mean difference in increase of Se concentration in maize grains between intervention and control groups was equal to 0.081 mg kg^{-1} (p-value < 0.001) or 4 µg kg^{-1} for each gram of Se applied as sodium selenate, corresponding to a relative difference of 94%, as shown in Table 27*Table 27*. Since soil Se uptake by maize crops, besides the Se

fertilization can influence Se concentration in maize grains, the study adjusted the mixed model for both the total and the KH_2PO_4-extractable soil Se concentration. However, this did not modify the effect of the intervention. The study therefore found a positive and significant effect of the biofortification intervention on Se concentration in maize grains.

For children, the mean difference in increase of Se intake from maize grains between intervention and control groups was 5.95 µg day^{-1} (p-value < 0.001), corresponding to a relative difference of 103%. In the case of women, the mean difference in increase of Se intake from maize grains between intervention and control groups was 16.35 µg day^{-1} (p-value < 0.001), corresponding to a relative difference of 94%. The study found a positive and significant effect of the intervention on dietary Se intake from maize grains.

The estimated average dietary Se intake from all foodstuffs consumed is presented for children in Table 25 for children and Table 26 for women. The actual Se concentration in the respective foodstuffs is presented in *Table 23*, and the estimated daily mean amount of foodstuffs consumed in *Table 24*. In the control group, mean Se concentration in maize (grains or ugali) between the baseline and post-trial was not significantly different (p-value = 0.85 and 0.79). Similar results were found for the other foodstuffs (p-value > 0.05). This suggested that time, i.e. the 6 months that separated baseline from post-trial, did not induce a significant change of Se concentration in consumed crops, and most importantly in maize. In addition, no significant difference in the average daily food intake amounts of all foodstuffs consumed by the population was observed between baseline and post-trial for both children and women (p-values > 0.05). Thus, the amount of food consumed, which is a key factor for computing the average dietary Se intake, appears to not vary over time, between the two stages of the intervention.

The mean difference in increase of average dietary Se intake between intervention and control groups was 10.95 µg day^{-1} (p-value < 0.001) for children, corresponding to a relative difference of 96%, and it was equal to 21.17 µg day^{-1} (p-value < 0.001) for women, corresponding to a relative difference of 108% (Table 27). The effect of biofortified maize grains on the average daily dietary Se intake was therefore positive and significant. Overall, Se biofortified maize grains improved the average daily dietary Se intake by 46% among children and by 44% among women. Compared to the control group, the treatment group was significantly more likely to have low risk of Se deficiency for both children (p-value = <0.001) and women (p-value = 0.001). The risk of dietary Se deficiency decreased in the treatment group from 89% to 39% for children and from 97% to 78% for women based on the EARs. Compared to the control, the percentage of the study population that had an adequate average dietary Se intake due to the intervention were 44% children and 22% women.

Table 23: Selenium concentration (mg kg^{-1} FW[a]) in different foodstuffs in Kiaga in the control group and the treatment group at baseline and post-trial, and significance of differences (p-values - Independent-Samples T-Test) within the control group and treatment group between baseline and post-trial

	Selenium concentration (mg kg^{-1} FW[a])								**Significance (p-values)**	
	Control group				**Treatment group**					
	Baseline		**Post-trial**		**Baseline**		**Post-trial**		**Within groups**	
Foodstuff	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Control	Treatment
Milk	0.018	*0.000*	0.018	*0.000*	0.018	*0.000*	0.018	*0.000*	0.99	0.99
Beef meat	0.241	*0.000*	0.241	*0.000*	0.241	*0.000*	0.241	*0.000*	0.96	0.94
Maize (grains)	0.021	*0.020*	0.015	*0.011*	0.019	*0.028*	0.118	*0.039*	0.85	<0.001
Maize (ugali)	0.013	*0.012*	0.009	*0.006*	0.011	*0.017*	0.070	*0.024*	0.79	<0.001
Rice	0.010	*0.010*	0.010	*0.010*	0.012	*0.013*	0.011	*0.012*	0.78	0.98
Bread	0.035	*0.012*	0.037	*0.012*	0.031	*0.012*	0.028	*0.010*	0.43	0.92
Millet	0.020	*0.011*	0.018	*0.012*	0.018	*0.011*	0.022	*0.010*	0.84	0.89
Sorghum	0.006	*0.002*	0.007	*0.003*	0.007	*0.004*	0.006	*0.004*	0.10	0.85
Bean	0.012	*0.009*	0.011	*0.008*	0.008	*0.005*	0.014	*0.013*	0.99	0.31
Green gram	0.015	*0.002*	0.015	*0.000*	0.013	*0.002*	0.013	*0.001*	0.87	0.99
Potato	0.004	*0.001*	0.005	*0.002*	0.004	*0.001*	0.005	*0.002*	0.60	0.52
Sweet potato	0.018	*0.001*	-	-	0.040	*0.021*	0.026	*0.019*	0.48	-
Green Banana	0.016	*0.009*	0.016	*0.010*	0.014	*0.011*	0.018	*0.009*	0.75	0.59
Kales	0.086	*0.024*	0.085	*0.008*	0.082	*0.009*	0.077	*0.012*	0.64	0.91
Spinach	0.014	*0.003*	0.013	*0.003*	0.010	*0.000*	0.010	*0.000*	0.75	0.39
Cabbage	0.011	*0.007*	0.013	*0.008*	0.009	*0.010*	0.009	*0.010*	0.57	0.98
Amaranths	0.080	*0.087*	0.066	*0.045*	0.047	*0.031*	0.048	*0.022*	0.55	0.97
Tomato	0.009	*0.007*	0.010	*0.006*	0.008	*0.006*	0.011	*0.005*	0.42	0.71
Onion	0.008	*0.000*	0.008	*0.000*	0.008	*0.000*	0.008	*0.000*	0.25	0.80
Carrot	0.020	*0.022*	0.025	*0.021*	0.045	*0.000*	0.029	*0.022*	0.61	0.99

[a]FW: Fresh weight basis. [b]-: foodstuff unavailable for sampling.

Table 24: Daily food intake (mean and standard deviation, g FW) for different foodstuffs for children and women in Kiaga in the control and treatment groups (pre- and post-trial)

	Children								Women							
	Control				Treatment				Control				Treatment			
Foodstuff	[a]Pre	*SD*	[b]Post	*SD*	Pre	*SD*	Post	*SD*	[a]Pre	*SD*	[b]Post	*SD*	Pre	*SD*	Post	*SD*
Milk	152	*120*	198	*119*	182	*128*	209	*124*	252	*169*	277	*174*	240	*133*	257	*148*
Beef meat	35.6	*15.3*	35.8	*20.4*	48.1	*26.9*	39.5	*39.0*	73.7	*34.7*	58.3	*27.6*	33.0	*15,6*	57.7	*43.3*
Maize (grains)	56.8	*50.0*	46.5	*32.0*	39.0	*43.3*	28.3	*35.5*	104	*69.9*	102	*70,0*	62.3	*41.0*	86.9	*83.0*
Maize (ugali)	72.4	*31.8*	110	*79.3*	93.1	*57.0*	134	*60.3*	194	*114*	248	*121*	247	*122*	241	*104*
Rice	105	*66.1*	151	*112*	138	*100*	177	*113*	249	*132*	246	*115*	233	*115*	280	*122*
Bread	52.8	*34.9*	90.9	*58.6*	89.6	*50.5*	77.2	*50.9*	119	*68.2*	98.6	*31.4*	93.6	*87.4*	93.3	*51.7*
Millet	7.97	*4.66*	4.96	*4.32*	5.26	*7.61*	4.62	*3.17*	9.40	*6.61*	7.15	*4.84*	11.7	*13.7*	11.4	*9.13*
Sorghum	6.45	*4.34*	2.61	*2.94*	4.92	*7.71*	2.44	*2.24*	6.32	*4.58*	4.58	*3.66*	6.98	*6.39*	6.91	*4.74*
Bean	67.3	*51.9*	75.3	*55.5*	81.7	*64.4*	78.0	*49.7*	139	*74.6*	109	*44.3*	141	*80.4*	96.7	*83.9*
Green gram	42.1	*24.9*	33.2	*3.6*	82.8	*44.4*	147	*81.3*	152	*88.6*	127	*72.5*	163	*19.8*	184	*158*
Potato	52.2	*36.3*	53.6	*38.4*	47.0	*40.1*	59.9	*36.1*	92.8	*56.8*	94.7	*45.8*	70.0	*45.6*	69.5	*51.2*
Green banana	67.8	*37.1*	93.4	*81.1*	45.0	*25.5*	114	*84.3*	154	*87.1*	-	-	186	*91.5*	137	*73.7*
Kales	28.7	*16.5*	35.4	*27.8*	19.2	*17.0*	40.2	*24.0*	52.6	*26.7*	58.5	*35.1*	50.3	*32.2*	54.6	*27.3*
Spinach	14.1	*5.40*	21.4	*9.70*	15.4	*7.40*	30.1	*20.2*	41.4	*24.5*	45.0	*21.4*	22.0	*5.90*	44.6	*18.0*
Cabbage	98.0	*41.2*	106	*73.2*	89.0	*13.0*	130	*46.3*	98.5	*72.6*	125	*65.1*	105	*98.1*	97.9	*62.1*
Amaranths	17.2	*10.4*	19.5	*10.9*	9.80	*11.5*	16.8	*13.9*	17.9	*11.2*	36.8	*24.1*	22.9	*22.3*	27.8	*29.7*
Tomato	44.6	*31.2*	33.9	*35.7*	38.3	*25.5*	42.6	*25.1*	56.7	*42.9*	57.8	*30.3*	67.5	*35.7*	61.3	*46.5*
Onion	7.90	*5.36*	10.8	*10.5*	8,87	*5.90*	9.42	*6.83*	11.0	*6.96*	12.0	*7.00*	14.8	*7.80*	11.4	*7.71*
Sugar	15.0	*11.7*	14.9	*7.7*	19.5	*10.4*	20.9	*10.8*	36.6	*28.3*	27.5	*21.8*	40.0	*22.8*	28.1	*15.2*
Tea leaves	0.53	*0.50*	0.76	*0.51*	0.94	*0.61*	0.90	*0.58*	0.91	*0.49*	1.66	*1,44*	1.50	*0.90*	1.44	*0.93*
Cocoa	0.38	*0.29*	0.73	*0.62*	0.85	*0.25*	1.64	*1.06*	1.39	*0.74*	2.38	*1.80*	2.56	*1.73*	2.76	*2.66*
Cooking fat	8.68	*9.28*	9.30	*10.6*	12.6	*12.1*	13.6	*8.71*	17.21	*13.38*	10.9	*7.31*	26.7	*12.9*	13.3	*9.03*
Salt	2.13	*1.49*	2.74	*2.69*	3.90	*3.35*	3.20	*2.12*	5.33	*5.43*	4.72	*4.61*	7.70	*4.49*	5.56	*4.05*

FW: Fresh weight basis. SD: standard deviation; [a]Pre: Baseline; [b]Post: Post-trial.

Table 25: Daily Se intake (mean and standard deviation, µg d-1 FW) from different foodstuffs for children in Kiaga in the control and treatment groups (pre- and post-trial)

	Daily mean Se intake (µg d^{-1} FW)								**Significance (p-values)**	
	Control				**Treatment**				**Within groups**	
Foodstuff	[a]Pre	*SD*	[b]Post	*SD*	Pre	*SD*	Post	*SD*	Control	Treatment
Milk	2.77	*2.19*	3.60	*2.18*	3.32	*2.34*	3.81	*2.26*	0.80	0.44
Beef meat	8.57	*3.68*	8.62	*4.92*	11.57	*6.47*	9.52	*9.38*	0.58	0.56
Maize	1.45	*1.50*	1.48	*1.23*	1.57	*1.48*	5.42	*4.09*	0.72	< 0.001
Rice	1.29	*1.91*	1.56	*2.97*	1.70	*2.45*	1.65	*2.16*	0.63	0.94
Bread	0.86	*0.58*	1.47	*1.26*	1.62	*1.42*	1.22	*0.97*	0.14	0.37
Millet	0.16	*0.16*	0.08	*0.06*	0.11	*0.20*	0.10	*0.08*	0.11	0.82
Sorghum	0.04	*0.03*	0.02	*0.02*	0.02	*0.02*	0.01	*0.02*	0.19	0.15
Bean	0.58	*0.45*	0.76	*0.83*	0.69	*0.77*	0.92	*0.97*	0.54	0.52
Green gram	0.67	*0.48*	0.48	*0.04*	1.03	*0.49*	1.94	*1.08*	0.54	0.23
Potato	0.25	*0.30*	0.24	*0.26*	0.21	*0.24*	0.27	*0.16*	0.92	0.50
Green banana	0.86	*0.53*	1.08	*0.58*	0.61	*3.46*	1.87	*1.71*	0.50	0.10
Kales	2.46	*1.65*	3.07	*2.55*	1.56	*1.40*	3.06	*1.79*	0.58	0.25
Spinach	0.21	*0.11*	0.26	*0.11*	0.15	*0.07*	0.29	*0.20*	0.54	0.25
Cabbage	1.00	*0.73*	1.46	*1.67*	0.77	*0.75*	1.30	*1.38*	0.46	0.58
Amaranths	1.44	*1.80*	1.12	*0.67*	0.41	*0.53*	0.81	*0.80*	0.58	0.16
Tomato	0.44	*0.42*	0.30	*0.27*	0.32	*0.32*	0.51	*0.40*	0.11	0.8
Onion	0.06	*0.04*	0.08	*0.08*	0.07	*0.05*	0.07	*0.05*	0.32	0.83
Sugar	0.16	*0.12*	0.16	*0.08*	0.21	*0.11*	0.22	*0.11*	0.79	0.70
Tea leaves	0.08	*0.08*	0.12	*0.08*	0.15	*0.10*	0.14	*0.09*	0.35	0.82
Cocoa	0.01	*0.01*	0.02	*0.02*	0.02	*0.05*	0.05	*0.03*	0.44	0.38
Cooking fat	0.03	*0.03*	0.04	*0.04*	0.05	*0.04*	0.05	*0.03*	0.77	0.91
Salt	0.03	*0.02*	0.04	*0.04*	0.06	*0.05*	0.05	*0.03*	0.57	0.46

[a]Pre: Baseline. [b]Post: Post-trial.

Table 26: Daily Se intake (mean and standard deviation, µg d^{-1} FW) from different foodstuffs for women in Kiaga in the control and treatment groups (pre- and post-trial)

	Daily mean Se intake								**Significance (p-values)**	
	Control				**Treatment**				**Within groups**	
Foodstuff	[a]Pre	*SD*	[b]Post	*SD*	Pre	*SD*	Post	*SD*	Control	Treatment
Milk	4.59	*3.08*	4.39	*2.43*	4.37	*2.42*	5.09	*2.88*	0.78	0.32
Beef meat	17.76	*8.37*	11.85	*6.07*	7.95	*3.75*	15.43	*11.34*	0.21	0.54
Maize	2.57	*1.57*	2.87	*2.64*	2.42	*1.22*	16.32	*10.60*	0.65	< 0.001
Rice	2.63	*2.34*	1.99	*1.92*	2.50	*1.51*	2.90	*2.77*	0.45	0.32
Bread	1.96	*1.28*	1.60	*0.95*	1.91	*1.04*	1.71	*1.22*	0.41	0.8
Millet	0.17	*0.11*	0.15	*0.13*	0.28	*0.16*	0.21	*0.15*	0.85	0.49
Sorghum	0.05	*0.04*	0.04	*0.03*	0.07	*0.02*	0.04	*0.02*	0.59	0.67
Bean	1.32	*1.19*	1.57	*1.01*	1.10	*1.06*	1.68	*1.50*	0.54	0.80
Green gram	1.84	*1.07*	1.93	*1.25*	2.17	*0.03*	3.06	*2.44*	0.94	0.49
Potato	0.44	*0.36*	0.37	*0.18*	0.28	*0.18*	0.29	*0.24*	0.47	0.30
Green banana	2.40	*0.52*	2.41	*0.55*	3.73	*2.29*	2.24	*1.42*	0.94	0.96
Kales	3.96	*1.96*	4.00	*2.64*	3.89	*2.79*	4.47	*1.95*	0.97	0.52
Spinach	0.59	*0.44*	0.52	*0.21*	0.25	*0.05*	0.47	*0.19*	0.70	0.57
Cabbage	1.17	*1.03*	1.28	*1.25*	0.52	*0.30*	0.80	*0.54*	0.80	0.23
Amaranths	2.06	*1.78*	1.86	*0.74*	1.09	*0.19*	0.65	*0.22*	0.87	0.14
Tomato	0.53	*0.46*	0.70	*0.58*	0.60	*0.55*	0.62	*0.56*	0.28	0.57
Onion	0.08	*0.05*	0.08	*0.05*	0.11	*0.06*	0.09	*0.06*	0.97	0.49
Sugar	0.39	*0.20*	0.29	*0.24*	0.42	*0.24*	0.30	*0.15*	0.80	0.83
Tea leaves	0.14	*0.08*	0.25	*0.23*	0.23	*0.14*	0.24	*0.15*	0.27	0.86
Cocoa	0.04	*0.02*	0.07	*0.05*	0.07	*0.05*	0.08	*0.08*	0.43	0.87
Cooking fat	0.06	*0.05*	0.05	*0.03*	0.10	*0.05*	0.06	*0.03*	0.28	0.30
Salt	0.08	*0.05*	0.07	*0.05*	0.12	*0.07*	0.08	*0.06*	0.73	0.62

[a]Pre: Baseline. [b]Post: Post-trial.

Table 27: Differences and mean changes in Se concentration in maize grains (mg kg-1), Se intake from maize (µg d^{-1}), and average dietary Se intake after intervention (µg d^{-1})

	Outcomes	n (cluster)	Differences at individual level ΔT (Mean, SD)	ΔC (Mean, SD)	Unadjusted effects at individual level Beta	95% CI	p-value	ICC (%)	RD (%)
	Se conc. maize	64 (6)	0.087 (0.049)	0.005 (0.028)	0.081	0.065 - 0.098	< 0.001	16	94
Children	Se intake from maize	59 (6)	5.96 (4.07)	-0.15 (1.94)	6.00	4.48 - 7.53	< 0.001	14	103
	Average Se intake	59 (6)	9.45 (14.46)	0.35 (6.60)	10.95	6.85 - 15.05	< 0.001	19	96
	Risk of Se deficiency	59 (6)	-50%	-6%	[a]44%	34% - 78%	< 0.001	21	114
Women	Se intake from maize	63 (6)	17.88 (10.41)	1.10 (3.83)	16.35	12.62 - 20.09	< 0.001	19	94
	Average Se intake	63 (6)	19.61 (17.03)	-1.65 (16.09)	21.17	14.88 - 27.46	< 0.001	12	108
	Risk of Se deficiency	63 (6)	-19%	3%	[a]22%	11% - 33%	0.001	5	86

n: sample size (control and treatment groups). ΔT: Post-trial minus baseline status in the treatment group. ΔC: Post-trial minus baseline status in the control group. SD: Standard deviation. CI: Confidence interval. ICC: Intracluster Correlation. RD: Relative differences. [a]: The risk of dietary Se deficiency in the model is a dummy variable: 0 for high risk (<EARs) and 1 for low risk (>EARs).

6.5. Discussion

Using a cluster randomized control trial, this study conducted an agronomic Se biofortification intervention on Se deficient farm households in Kiaga – central Highland (Kenya). Through an assessment of Se concentration in consumed foodstuffs and an understanding of the population's diet, we used difference-in-difference method to estimate the net change in the average daily dietary Se intake resulting from the Se biofortification intervention on maize crops, which was translated into a percentage decrease in risk of dietary Se deficiency. The biofortification of maize crops was found to have a significant positive impact on the average daily Se intake among the intervention group. After accounting for potential confounding factors such as soil Se concentration, the net impact of maize biofortification on average daily dietary Se intake was estimated.

At baseline, maize grains were characterized by low mean Se concentration. Based on estimated dietary intake data from Kiaga, maize was the most commonly consumed staple food, coupled with limited or no consumption of good Se sources such as fish, meat, and eggs. This explains the low dietary Se intake at baseline. The intervention successfully increased Se concentrations in the grains above 0.1 mg kg^{-1}, a level considered appropriate for dietary Se (Gissel-Nielsen et al. 1984). The increase in Se concentration in maize compares to previous studies. For example in Malawi, Chilimba et al. 2012 reported a Se concentration increase of 0.02 mg kg^{-1} in whole maize grain, per gram of foliar sodium selenate fertilization of maize crops. Wang et al. 2017 reported an increase in Se concentration in maize of 0.018 mg kg^{-1} for each gram of foliar Se applied in China. Mao et al. 2014 though reported a much lower increase in Se concentration in maize grains at 0.0086 mg kg^{-1} at a foliar fertilization dose of 1 g Se ha^{-1}, they recommended a Se application dose of 34 g ha^{-1} on maize crops, in order to achieve the biofortification target Se level 0.3 mg kg^{-1}.

In line with the literature, our study therefore showed that agronomic Se biofortification of maize crops significantly increases Se concentration in grains, and can therefore be considered as an effective strategy for Se deficient communities. When considering Se biofortification of other staple cereal grains in literature, we found a similar magnitude of impact on the Se concentration as was in maize grains. For instance, foliar sodium selenate fertilization at 20 g Se ha^{-1}, increased Se concentration in rice grain from 0.071 to 0.640 mg kg^{-1} in China (Chen et al. 2002). A foliar sodium selenate fertilization at 25 g Se ha^{-1} on upland rice increased Se concentration in the rice grain from 0.03 to 0.32 mg kg^{-1} in Brazil (Reis et al. 2018). For wheat, foliar sodium selenate fertilization at 30 g ha^{-1} increased Se concentration in wheat grain from 0.02 to 0.31 mg kg^{-1} in China (Wang et al. 2017). At a dose of 10 g Se ha^{-1} on winter wheat, it leads to a significant increase of the Se concentration from 0.032 to 0.445 mg kg^{-1} in the Slovak Republic (Ducsay et al. 2016). This highlights the importance of biofortification measures targeting the staple food of a population, enabling therefore more effective strategies.

Foliar Se fertilization avoids Se immobilization in the soil (De Temmerman et al. 2014). The technique is important especially considering the current climate change crisis, which is predicted to further decrease soil Se concentrations in agricultural soils, and hence exacerbate the prevalence of Se deficiency (Jones et al. 2017). The effect of climate change in terms of reduced rainfall and higher temperature results in oxidation of soil organic C-Fe and -Al associations that are responsible for the P adsorption in the soil, and hence leads to release of P and losses during runoff (Forber et al. 2018). The reduction in soil's P to Fe + Al ratio, which indicates how much Fe/Al is still available for Se sorption, increases Se adsorption (less competition for sorption sites), reducing Se availability for crops' uptake (Eich-Greatorex et al. 2010). In fact, the present study incurred unprecedented drought that resulted in inadequate rainfall at the time of foliar Se fertilizer application.

The average daily dietary Se intake at baseline was found to equal to 10.50 µg day^{-1} for children and 21.95 µg day^{-1} for women, corresponding to a risk of dietary Se deficiency of 90 % and 97% respectively. Extremely low Se intakes of <20 µg day^{-1} might result in clinical Se deficiency disorders (Fairweather-Tait et al. 2011). The women's average dietary Se intake is found to be much lower than previously reported dietary Se supply of 23 to 35 µg $capita^{-1}$ day^{-1} for the Eastern African region (see for instance Joy et al. 2014). However, the previously reported mean estimated risk of dietary Se deficiency in the region at 91 to 100 % (ibid) is relatively similar to this study's findings. A potential explanation to why average daily dietary Se intake in the current study is much lower than previously reported is due to overestimation of Se supply, as losses during food processing, preparation, household waste, and postharvest losses are unaccounted for when estimating dietary Se intakes based on per capita food supplies. An overestimation of dietary Se intake therefore results in an underestimation of the risk dietary Se deficiency. Availability of average dietary Se intake data and actual Se concentration of foods in this study eliminates such estimation errors.

The positive and significant effect of Se fertilization of maize crops at 20 g Se ha^{-1} on Se concentration in maize grains improved the average dietary Se intake from a control level of 10.92 µg day^{-1} to an adequate intake of 20.25 µg day^{-1} for children. This resulted in a 44% decrease in the risk of dietary Se deficiency. For the women, the average dietary Se intake increased from a control level of 19.38 µg day^{-1} to 34.76 µg day^{-1}. The 22% decrease in risk of dietary Se deficiency for women was therefore half that of children. This can be explained by the fact that women at baseline have a higher risk of dietary Se deficiency than the children. However, the percentage improvement in the average dietary Se intake for children (46 %) and women (44%) was comparable. In rural central Kenya highlands where households have limited resources to afford a diversified diet, children's diet is given priority. Scarce and seasonal foodstuffs such as milk, eggs, fruits, indigenous vegetables and some lentils are reserved for the children. Animal based products such as milk, eggs, fish powder are commonly added in snack meals for children e.g. in porridge, which locally serves as a snack throughout the day. This explains the lower risk of dietary Se deficiency among the children. Since there were no significant differences in observable factors of interest between the control and the treatment groups, and between the two phases of the experiment, we attribute the improvement of the average daily dietary Se intake in the study population to the Se biofortified maize grains.

The average dietary Se intake for women post-trial was 10.24 µg day^{-1} below the adequate intakes of 45 µg day^{-1} based on EARs. In human health, Se intake of 40 µg day^{-1} is needed for maximal expression of glutathione peroxidase - GPx, for protection against oxidative stress. Intakes less than this amount increase the risk of oxidative stress related diseases such as cardiovascular disorders and some cancers (Tapiero et al. 2003, Fairweather-Tait et al. 2011). As reported in Chapter 5, foliar Se fertilization of more than one staple crop in the central Kenya highlands i.e. for both maize and beans would achieve an adequate dietary Se intake, or a foliar Se fertilizer application on maize crops alone at a dose >20 g Se ha^{-1} in more Se deficient locations. However, the sporadic rainfall pattern has resulted in crop failure, with the bean crops being more adversely affected than maize crops.

Although the agronomic biofortification of staple crops as a population-based strategy can potentially increase dietary Se intake in Kiaga, biofortification of a single or few staple foods should be complemented by other existing measures such as dietary diversification. Reliance on few energy-rich foods is recognized as a major contributing factor to micronutrient deficiencies (Johns and Eryzaguirre 2006). Local communities in Kiaga raise cattle, goats, sheep, and poultry and hence, good dietary Se sources such as meat, eggs, and milk are locally available. However, they are sold rather than consumed (Bwibo and Neumann 2003). Considering the current climate change that exacerbates the prevalence of Se deficiency (Jones et al. 2017), diet diversification strategies aimed at promoting consumption of the locally available animal-based foods would play an important role in complementing the observed effects of the Se biofortification intervention.

This study was greatly affected by unfavourable weather conditions which resulted in changing the setting of the trial from one study location to another. As a result, the timing of the intervention which entirely depended on the local cropping calendar, was highly disrupted. As a result of drought, the trial was not conducted in Mbuyu, the study location that reported the highest risk of dietary Se deficiency. Accordingly, it should be noted that the findings of the intervention trial may not reflect the outcome in regions at higher risk of Se deficiency. In Kiaga, the alternative high risk region, only six villages were eligible for the trial since the maize crops were not adversely affected by the drought. This reduced the power of the study as only three villages were allocated per trial arm. These findings therefore need confirmation. This study provide useful estimates to design a larger study with potentially more objective outcomes i.e. using more sensitive biomarkers. Furthermore, Se fertilization was conducted under dry weather conditions against the recommended Se fertilization during a rainy season and on a sunny day. This means that the outcome of the biofortification intervention may not be representative for the full impact under good weather conditions. Moreover, biofortification of bean crops which responds better to Se fertilization than maize was curtailed by the drought. Accordingly, the impact or the potential of the Se biofortification intervention in improving the dietary Se intake may have been underestimated.

The study therefore recommends further research based on dietary intake data for a longer a period in order to factor in the annual differences in dietary intake due to the unpredictable rainfall pattern. For the rural farmers the rain-fed agriculture is hit by climate change. Food production varies a lot each year as the weather no longer seems to follow the usual seasons of short and long rains. Exposing the treatment group to the biofortified staple grains for several years will enable drawing conclusions on long-term effects on health and nutritional status. This would also increase chances to biofortify other staple crops such as the beans during seasons of favourable weather conditions. Larger studies would also confirm the findings of the study and more so using more sensitive Se status assessment such as blood analysis.

CHAPTER 7: GENERAL DISCUSSION AND CONCLUSION

This final section highlights the major results and contributions of this PhD project and reflects on the main outcomes of the thesis. It also highlights limitations of the conducted research and needs for further investigation.

7.1. Contribution to management of Se deficiency in developing world

7.1.1. Expanding knowledge on Se deficiency

Policy makers in the developing world have been largely unaware of Se deficiency as a potential public health issue. In the past, most studies addressed Se deficiency 'in parts', focusing for instance on the identification of Se deficiency in soils or on the Se status of a particular population. Despite the urgency of the issue in developing country settings, the literature shows a critical gap in tackling the Se deficiency from an integrative perspective. Indeed, Se intake of the population is rarely considered as part of the public health policies, nor are the type of management practices of arable soils, which could contribute to increasing the uptake of Se by crops. This is particularly problematic in rural subsidence farming contexts where households depend primarily or exclusively for decades on a single parcel of land to meet their food and nutrient requirements. These challenges are due to lack of representative data for the rural areas that suffer most from Se deficiency, and to the cost of analysing Se content. It is also linked to the complexity of the problem, which necessitates a comprehensive understanding of the nutrient journey from the soil to the crops, to the diet, and finally to the body or health. This includes the understanding of various mechanisms influencing the presence of Se in the soil, its availability for plants, the uptake of Se by crops, the consumption of these crops by the households, and the assimilation of Se in the body. Understanding the multiple factors causing Se deficiency within the food system is vital in targeting and prioritizing among the possible intervention measures and policy instruments. This calls for the need to have a "full picture" evaluation for assessing the effectiveness of various agricultural strategies and interventions on the average Se dietary intake in a population.

This thesis contributed to a better understanding of the issue of Se deficiency in populations relying on rain-fed subsidence farming. Using an agricultural-nutritional-environmental chemistry perspective, the research conducted a unique and comprehensive assessment of Se status from the soil, to the crops, and to the population. This assessment was carried out in an area characterized by the absence of long-term strategies to reduce the risk of Se deficiency in the population, and considering a variety of conditions. First, different soil locations were selected representing various soil geochemical characteristics as those are likely to influence Se content in the soil and its uptake by crops. Second, Se status in the populations was investigated for both children and women, which are the two profiles most vulnerable to nutrient deficiencies. The thesis therefore provided insights in the status of two age ranges varying in their dietary and nutrient

requirements and in their assimilation potential of the nutrient. Third, a very large variety of available foodstuffs was considered, not only considering the most consumed foodstuffs.

The integrated analysis conducted in this thesis provides therefore on the one hand new knowledge on the issue of Se deficiency and the distribution of Se deficiency risks across rural developing world, and on the other hand a more complete framework of analysis that could be applied to other types of nutrients essential to the body across other settings/locations. This knowledge will facilitate the design of integrative and sustainable mineral-deficiency interventions suitable for rural subsistence farming settings to tackle ”hidden hunger”.

7.1.2. Provision of new evidence of Se deficiency

The majority of morbidity and mortality cases in the developing world is due to lack of early diagnosis and timely treatments. In such a scenario, biomarkers are an indispensable resource as indicators of biological processes, specific disease conditions, or response to interventions (Gupta et al. 2014). Data on Se status or deficiency and the underlying explanatory mechanisms contributing to Se deficiency are key in designing suitable interventions to avert the adverse effects on health. However, in the developing world such as in Kenya, numerous factors cumulatively hinder biomarker sampling and analysis ventures. These include economic crunches, lack of awareness and education, lack of biorepositories, enormous diversities in socio-epidemiological background, ethnicity, lifestyle, diet, exposure to various environmental risk factors and infectious agents, and ethical and social issues (Gupta et al. 2014).

Previous studies in developing countries have mainly relied on per capita food supplies from food balance sheets and food composition tables as sources of nutrient intake information. These sources are recognized to overestimate nutrient supply as losses during food processing, preparation, and household waste are not accounted for, and this results in an underestimation of the risk of micronutrients deficiency. Accordingly, more reliable data are needed. The above challenges withholding, this thesis assessed Se status of the target population using hair samples and estimated the risk of dietary Se deficiency based on average dietary Se intake.

This research project conducted an in-depth and unique assessment of the Se status in Kenya. It generated reliable dietary intake research data through estimating (currently lacking) average Se dietary intake of the population, and collecting actual Se and other mineral concentration data for the whole range of locally consumed foodstuffs. The thesis contributes therefore to fill an important gap in the evidence of this health issue and to feed into the incomplete Se database. Findings related to the Se content of the various foodstuffs analysed in our research will indeed be published to complement local food composition tables and therefore substantially increase accuracy of future assessments. Moreover, the thesis provides evidence that hair Se concentration is a sensitive and reliable indicator for dietary Se intake. The risk for dietary Se deficiency of the target population is indeed reflected in their hair Se status. With the installation of ICP-MS equipment

capable of detecting trace levels of Se in the lower microgram per gram (ppm) or nanogram per gram (ppb) range in the hair, Se status of women and children in this study was compared with their actual risk of dietary Se deficiency. The lack of precise data on agricultural soils' nutrient content makes the design of policies addressing Se deficiency particularly complex. This research demonstrates that an in-depth assessment of the key elements contributing to Se deficiencies in individuals and their relationships, including diet, Se content of food consumed, and soil Se status, can contribute to development of a suitable intervention.

7.1.3. Uncovering the factors contributing to Se deficiency in the rural developing world

Food systems need to produce adequate dietary Se intake i.e. at least 40 µg day^{-1} for adults, to support the maximal expression of the GPx-3 (Combs 2001). However, as observed in this thesis, the average daily dietary Se intake for women is found to be lower than the required Se intake level of 40 µg day^{-1} in the study locations assessed. Moreover, extremely low Se intakes of <20 µg day^{-1} are also observed in Mbuyu. However, because the Se deficiency data are scarce for the developing world, its impact on human health remains unknown, and hence there is a need to extend the research on adverse health effects that may be related to Se deficiency in developing countries. In literature, Se deficiency is mainly attributed to low soil Se phytoavailability leading to low Se concentration in food, and is aggravated by diets subsisting on cereals with limited consumption of animal source foods (White and Broadley 2008, Welch and Graham 2005). As was observed in this thesis, the poor Se status of agricultural soils and low dietary diversification are key factors contributing to the widespread Se deficiency, among other potential factors discussed below.

This thesis embarked on first providing the missing soil Se status data for various agricultural soil types in Kenya that differ in geology, relief, and climate. Generally, it was found that the mean total soil Se concentration in both the Central Kenya Highlands (0.465 mg kg^{-1}) and the Lake Victoria Basin (0.394 mg kg^{-1}) are below the agricultural soil's Se deficiency threshold of 0.6 mg kg^{-1} (Fordyce 2013), coupled with an extremely low KH_2PO_4-extractable soil Se concentration of 0.008 mg kg^{-1} for the Central Highlands and 0.009 mg kg^{-1} for the Lake Basin. This may explain the low Se content in local foodstuffs in both regions, and consequently, the 100% risk of dietary Se deficiency in most study locations. Thus, the thesis confirms that the lack of Se status data for developing countries is not a basis to rule out existence of Se deficiency. More research is however needed to explore on the health implications of the observed risk of Se deficiency.

The thesis further highlighted the key geochemical characteristics influencing Se status of local staple foods including soil pH, Fe, Al, Mn, P, and S contents. Across the study locations, the soil pH ranged from moderately acidic to neutral. With the soil Se species largely depending on pH and Eh (Mayland et al. 1991, Wikipedia 2019), selenate predominates in alkaline soils, while

selenite exists in acidic soils (Gupta and Gupta 2017). At an Eh within the range of 0.4 to 0.8V, soil pH induces transformations between selenite and selenate, i.e. at higher redox potential and pH, the Se oxidation state is Se^{6+} (selenate). This selenate is less adsorbed by soil minerals and is therefore available in the soil solution, and taken up by crops. However, at lower pH, the oxidation state is Se^{4+} (selenite) and hence, Se is chemically more active. Selenite is adsorbed by ligand exchange onto soil clay surfaces by Fe, Al, and Mn oxides, with greater affinity than selenate. As a result, Se availability is limited in the soil solution (Blaylock and James 1994). However, the same oxides and particularly the Fe/Al oxides form stronger bonds with PO_4^{3-} anions. An increase in soil PO_4^{3-} has been shown to increase Se uptake by plants as the PO_4^{3-} ion is readily adsorbed in soils, which displaces selenite from fixation sites making it more available in the soil solution for uptake by plants. However, increasing the levels of PO_4^{3-} in soil can dilute the Se in the vegetation by inducing increased plant growth (Jacobs 1989, Maryland 1994, Neal 1995, Beauchemin and Simard 1999, Dhillon and Dhillon 2000, Eich-Greatorex et al. 2010). This may explain why study locations in Central Highlands such as Njoune and Ruiri with the highest P to Fe + Al ratios have higher Se concentration in foods, as compared to locations with the lowest P to Fe + Al ratios such as Marimanti and Mbuyu, which recorded the lowest Se concentration in foods. The P to Fe + Al ratio indicates how much Fe/Al is still available for Se sorption. Notably, the P to Fe + Al ratio is highest in the Central Highlands, as compared to the Lake Basin. The soil Fe/Al oxides are therefore more saturated in the Central Highlands resulting in less Se sorption to the soil and consequently, higher Se uptake by crops. Accordingly, a higher Se availability is expected in the Central Highlands. However, considering that the KH_2PO_4-extractable soil Se is similar in both regions, the lower soil S content in the Lake Basin may also explain the higher Se concentrations in some staple foods such as maize. The presence of SO_4^{2-} anions influences Se uptake by plants by competing for fixation sites in plants' roots. SO_4^{2-} inhibits the uptake of Se by plants and has a greater effect on selenate than selenite. This is mainly because selenate uptake occurs via a sulphate transporter in the root plasma membrane (Maryland 1994, Neal 1995). A low soil S concentration therefore indicates less competition of S with available/soluble Se for plant uptake. Generally, Se partitioning in the soil involves an interaction of multiple factors that affects the final Se concentration in the crop. These include not only the presence of SO_4^{2-} and PO_4^{3-}, soil pH and redox potential, and content of sesquioxides and clay, but also presence of organic matter (OM), microbiological activity, rainfall, among others, as broadly discussed in Chapter 1. Such data on geochemical characteristics inhibiting or enhancing soil Se availability and hence Se concentration in foods form the basis under which soil-based agronomic interventions can be designed i.e. addition of Se to the soil or modification of inhibiting soil characteristics or both. Soil application necessitates a form of Se assimilated by plant roots, plus factoring in the physiological limitations to the supply and phytoavailability of mineral elements in the rhizosphere solution (White and Broadley 2009).

Second, developing countries have in the last decades experienced a shift in diets (Stamoulis et al. 2004). The dietary pattern has shifted away from traditional diets towards more "westernized" ones. This is accompanied by changes in food production, retailing, and distribution. This dietary shift has important implications on rural food security, food safety/quality and consequently, nutrient deficiencies and associated diet-related non-communicable diseases. These changes are more rapid and evident in developing countries experiencing rapid economic development and transformation such as in Kenya (Miller and Yeager 2018). The resulting nutrition transition is characterized by a shift in staple foods that includes reduced consumption of course grains, such as millet, sorghum, and tubers, and increased consumption of maize flour, wheat flour, and polished rice (OECD 2006, Dapi et al. 2007). As observed in this thesis, the major staple foods in both the Central Highlands and the Lake Basin are maize, rice and wheat grains. Moreover only two tubers are consumed in scaled amounts i.e. potato in Central Highlands and cassava in the Lake Basin. This shift in staple foods is of concern because the mineral content differs considerably among staple foods. As observed in this thesis, Se concentration is indeed lower in maize (0.169 mg kg^{-1}), rice (0.046 mg kg^{-1}), and wheat (0.056 mg kg^{-1}), compared to Se concentration in millet (0.214 mg kg^{-1}) and sorghum (0.234 mg kg^{-1}). The nutrition transition is characterized by reduced micronutrient density in diets, which are not compensated for by increased amounts of the adopted staple foods (Tilman and Clark 2014). Notably, millet and sorghum which are more drought resistant and hence are potential dietary Se sources in the face of the current climate change, are only consumed in very minute amounts as ingredients for children's porridge. Among the nine locations studied in this thesis, millet and sorghum is only cultivated in Marimanti as the main staple food. This is mainly because the sandy soils and extreme dry weather conditions in the region do not support maize cultivation. Farming diversity in terms of cultivation of millet and sorghum as staple crops besides maize can diversify the diet and hence improve Se intake.

We observed an overreliance on diets of low biodiversity and dietary diversity in across the study locations. As a result, inadequate dietary Se intake and high risk of dietary Se deficiency is observed at all study locations. The diet is characterized by a low food biodiversity of 7 food species for women and 8 food species for children, and on average 4 food groups per day. Thus, the thesis demonstrates that a low dietary diversification also contributes to the dietary Se deficiency among the rural population in Kenya.

Third, information gaps at the downstream end of the food system (affected rural population) also contribute to inadequate micronutrient (Se) intakes (Brouwer et al. 2011). As observed in this thesis and in line with literature, good Se sources including meat and eggs are produced as rural subsistence farmers mainly practice mixed farming, yet, these foods are sold rather than consumed. Almost every household raises either cattle, goats, sheep, or chicken, but little meat or egg is consumed in the daily diet. The local diets are instead based on cereals, legumes, and tubers (Bwibo and Neumann 2003). Although the linkage between nutrition knowledge and Se deficiency is not measured in the thesis, Se deficiency is a form of "hidden hunger" that does not

manifest itself in obvious sensation unlike for the energy or protein deficiency that stimulates physical sensations that trigger food consumption. Thus, the rural population is virtually unaware that they lack adequate Se in their diets, besides its health implications. This information gap contributes to disregard of a diversified diet in all study locations, as was observed during the 24HR recall interview. As evident in literature, nutrition education intervention so far focuses on macronutrient rather than micronutrient intake, and is located within the developed rather than developing world (Poelman et al. 2013). Indeed, existing pre- and post-natal education and promotion of breastfeeding and appropriate complementary feeding practices for young children are in line with awareness creation to reduce Se deficiency. Nutrition education intervention would therefore provide the unavailable data needed to assess the linkage between nutrition knowledge and micronutrient deficiency, which remains unexplored. However, it should be noted that education alone will probably not be sufficient to achieve a wide uptake of Se biofortification strategies.

Fourth, another important factor contributing to Se deficiency is the on-going climate change, and resulting fluctuation in relative accessibility to food groups by the affected rural population. Of interest to this thesis, climate change indirectly contributes to Se deficiency by the decline in diet diversity as farming diversity narrows down to few draught-resistant crops. According to Jarvis et al. (2006), degradation of the ecosystems due to climate change have adversely affected agrobiodiversity. Consequently, the contribution of plant and animal diversity to food security and dietary quality has drastically declined resulting in undernutrition (Walingo et al. 2009, Maitima 2010). In such cases, education and outreach on environmental conservation aimed at stopping the ongoing ecosystem degradation, and subsidies for population-based approaches involving food biofortification, may be policy instruments of choice (Graham et al. 2007).

The findings highlighted above call for the need to address Se deficiency through a combination of agricultural- and nutritional-focused campaigns and policy measures. This translates on the one hand to the design of intervention measures for overcoming the Se deficiency, through population-based approaches including food biofortification and improved soil management. On the other hand, this means to raise awareness in the affected rural population on the importance of a diversified diet in terms of composition and variation. This includes increasing dietary diversification such as the consumption of locally available animal-source foods, and expanding the range of consumed staple foods. While the economic limitations of the rural communities may hinder access to a high dietary diversification, Se agronomic biofortification of local staple crops would complement the goal towards an adequate dietary Se intake.

7.2. Contribution to the design of interventions for eliminating Se deficiency in the rural developing world

7.2.1. Addressing Se deficiency in the rural setting

The key factors contributing to the Se deficiency across the study locations are Se deficient agricultural soils and a low diversified diet. Whereas iodization of salt and vitamin A supplementation has been highly effective, in developing countries, increasing dietary diversification and/or Se agronomic biofortification are potentially effective interventional strategies to address Se deficiency among affected rural communities as discussed below.

Increasing dietary diversification is recognized as the most reliable way to provide a wide range of micronutrients (FAO 2009). However, its achievement requires adequate supply, and continuous access and consumption of food varieties within and between the food groups. Since the rural communities are subsistence farmers, diversifying rural food production can be a useful approach to improve diet diversification. For instance, in view of the current drought and high temperatures brought about by the climate change, a wider cultivation of the more drought-resistant cowpeas, which are currently only cultivated in a few areas in the central Highlands, can be introduced to replace beans, and the more drought-resistant millet and sorghum can be cultivated more to accompany maize as staple foods. These crops are not only more drought resistant, but they also have higher Se contents. However, Sibhatu et al. (2015) report that increasing on-farm diversity is not always the most effective way to improve dietary diversity in smallholder households. They observed that a high food production does not necessarily mean a high consumption diversity. This can be explained by the production of similar food crops across the country resulting in overproduction. Seasonal flooding of foodstuffs causes low market demand (no/low returns for farmers), while most of the produce ends up in postharvest losses due to poor storage facilities, lack of transport infrastructure, and the absence of value addition techniques. Sibhatu et al. (2015) further observed that access to the market (food purchase) has larger positive effects on dietary diversity than those of increased production diversity and hence, the market transactions reduced the role of farm diversity for household nutrition. Yet, this option is only possible for people with higher incomes, who can afford to diversify their diet through market purchases or through farm diversity discussed above. However based on the regression projection conducted in this study, approximately 8 additional food species or 4 food groups per day would be required in order to achieve an adequate dietary intake at the population level. Such objective is difficult to reach due to the low income, food production, and farming diversity that is characteristic to rural areas of the developing world. About half of the Kenyan population mainly in rural areas, is unable to afford sufficient and diversified diet to meet recommended daily requirements (World Bank 2009), which results in the dependence on diets based on few staple foods produced from their subsistence farms. Other factors besides economic conditions influencing food accessibility include agricultural

practices (mono-cropping and continuous cropping without nutrient replenishment), high farm-inputs costs, climate change, and illiteracy (Bain et al. 2013). Improvement of nutritional outcomes therefore depend on a combined support comprising of raised income, expansion of nutrition supplementation coverage for vulnerable population groups, a more nutrition-sensitive agriculture, and ensuring food availability and stability (Townsend 2015). It is recognized that population-based approaches involving food biofortification, along with food security, and nutrition education are the most effective way to safely meet community health needs (Tulchinsky 2010).

Furthermore, implementing a diversification strategy in these contexts necessitates to reformulate existing food and agricultural policies that largely emphasize on primary agricultural production, and do not include micronutrients outcomes in its goals (Tontisirin et al. 2002). For instance, increasing fish intake in the Central Highlands (92% of total domestic fish supply comes from Lake Victoria) would be challenged by the significant drive to sell fish overseas, resulting in reduced availability to the local community (Reuben et al. 2006). In addition, overexploitation and environmental pollution of the lake from water hyacinth and agrochemicals have substantially affected the supply of wild capture fish, explaining the decline in fishery resources. Over the last decades, fish landings have declined by 80% for Nile perch and by 60% for tilapia (Quagrainie et al. 2009). Considering high fish prices and transportation cost (distance of 350 km between the two study regions), introducing fish intake in the Central Highlands would be difficult to implement without necessary policy formulation. However, increasing consumption of legumes and leafy vegetables in the Lake Basin would be more easily feasible since the production of legume varieties in the Central Highlands exceeds local needs. However, as reported by Njoroge et al. (2019), domestic consumption of such grains is in competition with exports and postharvest losses with 80% of farmers facing pre-drying losses due to insects (48%), rodents (40%), and birds (39%). Additional postharvest losses occur during grain storage due to insect (57%) and rodents (43%). Considering the above challenges, increasing dietary diversification in the Lake Basin would require solutions for pest infestations in the field and improved storage technologies in the Central Highlands.

In regard of these various challenges, a plant-based Se biofortification strategy may be a more suitable strategy in the contexts studied in this thesis. This should be combined with the introduction of the drought resistant crop varieties such as cow peas, millet and sorghum. The thesis tested the plant-based Se biofortification strategy by increasing Se concentration in staple grains, and applied it to address the risk of dietary Se deficiency in the Central Highlands. According to Garg et al. (2018), this is a promising, cost-effective, and sustainable strategy to deliver micronutrients to a population that has limited access to diversified diets and lack micronutrient interventions. Moreover, agronomic biofortification is increasingly being adopted in developing countries (Bouis and Welch 2010), where it has proven to be feasible as exemplified by the success of Se fertilization of crops in Finland (Aro et al. 1995), zinc fertilization in Turkey (Cakmak et al. 1999), and I fertilization in irrigation water in China (Jiang et al. 1997).

Generally, the thesis concludes that increasing dietary diversification may be more feasible in rural areas at risk of Se deficiency where intake of a variety of plant-based foods can be achieved and complemented by intake of good Se sources such as fish, meat, and eggs. Selenium agronomic biofortification is more adapted to rural areas where increasing dietary diversification is not applicable, but dietary diversification can be complemented with an increased Se concentration of well selected foods through Se fertilization of respective crops (Ros et al. 2016).

7.2.2. Consideration of the local context in designing Se biofortification intervention

A major outcome of this thesis resides in the design of a Se biofortification intervention, and the evaluation of its potential impact on the risk of dietary Se deficiency among the affected rural communities. To achieve this, the Se biofortification experiments (for crops' response to Se application doses and techniques) and intervention trials were based on a community-participatory approach. The approach involved engaging the affected communities who are mainly subsistence farmers, their households, and respective parcels of land in all phases of the research. This approach aided in identifying local food resources and dietary intakes. This way, limiting factors to the use of fertilizers by local farmers, such as lack of agronomic guidance and economic constraints, were also identified.

The thesis first analyzed soil geochemical characteristics of the local farms on all study locations. The approach provided the opportunity to enlighten the farmers on why and how their farms suffered nutrients depletion following years of continuous cropping without nutrient replenishment. The thesis demonstrated that addition of P and N fertilizer (DAP) resulted in a positive effect on Se concentration in maize and bean grains, through an increase in Se mobility and availability. This is likely due to the added phosphate ions going into competition for sorption sites and hence, the applied Se remains in the soil solution for plant uptake. Notably on one hand, an increase in soil PO_4^{3-} content induces plant growth and hence root development that increases Se uptake by crops. However on the other hand, the contents may be diluted in the crop by the increased biomass production. This explains the lack of significant effect of addition of P in Mbeu and Kiaga with relatively higher soil P content. On such locations, addition of P will have no impact on soil Se bioavailability as all the sorption sites are already saturated with P. However in Mbuyu, the location with the lowest soil P content, the effect of adding P on soil Se bioavailability due to the P displacing Se from fixation sites supersedes the neutralization of the increased Se uptake by the increase in biomass. This is because the margin to saturate sorption sites in Mbuyu with P is wider, which translates to more Se desorption to the soil solution. In general, although Se is reported to have no effect on crop's yield (Chilimba et al. 2012, Wang et al. 2013, Mao et al. 2014), supplementing locally available fertilizers (i.e. DAP) with Se would result in both increased Se concentration in grains and yields, which can aid in convincing the farmers to adopt biofortification practices.

However, we acknowledge that this would not be affordable to most of the small-scale farmers. Currently, the use of fertilizers by subsistence farmers varies from region to region, largely depending on the reliability of rainfall patterns or access to water for irrigation such as farmers whose farms border a permanent river. In study locations such as Kibirichia which has very reliable rainfall pattern or some parts of Kiaga that have access to water for irrigation, small-scale farmers commercialize their farming and are therefore able to predict the output benefits of using fertilizers. However, for small-scale farmers in arid areas such as in Mbuyu and Marimanti, who depend on rain-fed agriculture, fertilizer application is considered a risk with the current unpredictable rainfall patterns. Generally, with or without resources, rural dwellers trade off between producing enough foods, which can include the costs of such fertilisers, or buying foods which is equally expensive. The population group that does not fall under these two categories end up starving without food aid intervention.

Moreover, the recommended Se biofortification doze of ~10 g ha^{-1} for food production was used (Ros et al. 2016). Furthermore, half this recommended dose (5 g Se ha^{-1}) and double the dose (20 g Se ha^{-1}) were tested. A safe fertilization rate was chosen particularly because the biofortification trials were conducted on lands farmed for production of food that consists of the biggest portion of the household diet. There was therefore no room for testing a dosage that could imply a risk for over-exposure of a number of households. The locals had expressed concerns at the start of the research on their parcels of land to be used for the Se biofortification trials. They were worried that this would lead to a decline in their annual harvest, on which they depend for their food supply. This was even more the case in high Se deficiency risk regions experiencing low soil and air temperatures such as in Mbuyu, as those communities have only one harvest season per year.

The Se biofortification trials were in addition conducted on a variety of agricultural soils and under the prevailing climatic and farming conditions i.e. rainfed (no irrigation), with usual land tillage and soil conditions and no use of pesticides or herbicides. The results from the biofortification trials therefore present actual crops' response to Se fertilization under "real life" conditions. The biofortification trials and intervention tested in this research make a substantial contribution to inform and predict the potential effects of biofortification in the rural context.

While matching the Se biofortification design to the local context is demanding, it has the benefit of being more convincing to policy makers. It shows a higher level of understanding of the complexity of the issue, which is crucial to the development of suitable policies. Thus, the findings may have higher chances to be taken on board for the design of policy measures. In addition, community participatory approaches aid in identifying nutritional, agronomic, and economic needs of the vulnerable rural communities, which aid in setting the targets for the Se biofortification as an important strategy to alleviate Se deficiency.

7.2.3. Consideration of multi-mineral deficiencies in rural areas: cross-complementarities

This research found that both soil and foliar applications of sodium selenate fertilizer significantly increased the Se concentration in both maize and bean grains, with foliar application techniques potentially being more effective. In addition, the thesis further tested for the potential inclusion of Zn and I, which are often lacking in rural diets in the Se agronomic biofortification of maize and bean crops. This was following successful increase in foods' content of these minerals through independent agronomic biofortification treatments. However, exploration of a simultaneous biofortification intervention to address the three mineral deficiencies simultaneously is lacking, especially in the context of Sub-Saharan Africa. The goal was therefore to test if Zn and I fertilizers affect the crops' response to Se fertilization. Notably, suitable interventional measures consider that no single nutrient deficiency can be addressed in isolation because it exists together with other forms of malnutrition. It was found that the addition of Zn and I did not affect the effect of Se fertilization on Se concentration in grains. These findings indicated that an agronomic biofortification strategy can be applied to simultaneously address Se, Zn, and I deficiencies in the affected rural areas. In literature, this strategy was also found to effectively increase the concentration of each of the three mineral elements both through independent treatments (White and Broadley 2009) and also in combination (Mao et al. 2014). The respective mineral concentrations in the biofortified food upon combined biofortification were identical to the concentrations found upon individual Se, Zn, and I treatments. This finding is important because in human nutrition, micronutrient status suffers or benefits from cross-complementarities between nutrients, i.e. deficiency in one mineral element may inhibit absorption of another mineral or worsen the effects of another mineral deficiency (Sahn 2015). Of interest to this thesis, Se deficiency for instance is hypothesized to exacerbate the harmful effects of iodine deficiency on the thyroid (Arthur et al. 1999), neurological cretinism may results from iodine deficiency while Kashin Beck disease may results from Se deficiency. Since both deficiencies are hypothesized to result in abnormal gait or bone disorder, it seems likely that myxoedematous cretinism stems from combined Se and I deficiencies i.e. exacerbates hypothyroidism (Vanderpas et al. 1990). Considering the complex multi-mineral deficiency problems in the rural developing world, a simultaneous Se, Zn, and I agronomic biofortification will not only address the individual mineral deficiencies, but it may also reduce the negative impacts of widespread Fe deficiency among the affected communities. This is because first, Se has a normalizing effect on Zn and Fe in the body, i.e. it increases the concentration of Zn and Fe at key sites such as erythrocytes when these elements are deficient, and reduces potentially harmful high Fe concentration in the liver during infection (Lyons et al. 2004). Second, Graham et al. (2012) argued that much of the current Fe deficiency in the world may be due to underlying Zn deficiency. Iron status depends on both Fe consumption and Fe uptake. However, Fe absorption is regulated by a molecule called hepcidin, whose synthesis is induced by infection and inflammation. Zinc deficiency on the other hand

aggravates oxidative stress in cells causing systematic intestinal inflammation, contributing to reduced Fe absorption by inducing hepcidin synthesis, which also increases Se requirements for protection against the oxidative stress (Tapiero et al. 2003). Zinc deficiency therefore exacerbates both Fe and Se deficiency (Graham et al. 2012). As is the case in the Central Kenya Highlands, Fe, Zn and Se deficiency often co-occur in African regions having highly weathered acidic soils. Therefore, a simultaneous Se, Zn, and I agronomic biofortification intervention strategy, complemented with dietary diversification, and nutrition education, will potentially improve dietary Se intakes of the rural developing world.

7.3. Effectiveness of Se biofortification intervention in addressing the risk of dietary Se deficiency

7.3.1. Suitable Se biofortification rate and technique

The Se biofortification intervention was aimed at addressing dietary Se deficiency risks in the rural Central Kenya Highlands. The cluster-randomized controlled trial was based on the findings from biofortification experiments in locations identified at high risk of Se dietary deficiency (Chapter 5). The appropriate Se fertilization dose and technique was selected based on the following key findings. First, compared to soil fertilization, foliar Se fertilization was found to be more effective in increasing Se concentration in grains, i.e. resulting in 6 and 6.7 times higher concentrations in maize and bean grains, respectively. Foliar fertilization was therefore the Se fertilization technique of choice. Second, considering the effect of foliar Se fertilization on the average dietary Se intake (based on estimated dietary intake data), foliar Se fertilization of 10 g ha^{-1} on both maize and bean crops successfully improved Se intakes to adequate levels of 27 µg µg day^{-1} for children and 54 µg day^{-1} for women. If only bean crops are biofortified at the same rate, dietary Se intake would still be adequate at 25 and 51 µg day^{-1}, respectively. However, biofortifying only maize would result in an inadequate dietary Se intake. Therefore, fertilizing only beans is more efficient compared to fertilizing only maize. However, the current inadequate rainfall and high temperatures due to climate change have adversely affected crop production in the Central Highlands, and bean crops have been more affected than maize. In this regard, in the subsequent biofortification intervention trial only maize crops were biofortified. As a possible response to climate change, farming diversity aimed at substitution of maize with millet and sorghum and/or beans with cowpeas, which are more drought-resistant crops would result in higher Se intake.

According to results from the biofortification experiments and average dietary data from the study locations, a foliar application of 20 g Se ha^{-1} on maize crops would result in an average dietary Se intake of 16 µg day^{-1} for children and 33 µg day^{-1} for women. Though slightly below adequate Se intake levels, this Se fertilization dose was considered suitable for the intervention trial. However

in general, we conclude that, during good weather conditions supporting proper growth of both maize and beans (intercropped in rural areas), a foliar Se fertilization of 10 g ha^{-1} is suitable, and the same application dose in a case scenario where only bean crops are biofortified. When only maize crops are to be biofortified, a foliar Se fertilizer application dose of at least 20 g ha^{-1} is suitable. However, in areas at higher risk of dietary Se deficiency such as in Mbuyu, a dose > 20 g ha^{-1} on maize crops is more suitable. Based on the estimated dietary intake data of the Mbuyu region, an extrapolated Se application dose of 31 g ha^{-1} is necessary to achieve adequate average dietary Se intake.

7.3.2. Impact of Se biofortification intervention on the risk of dietary Se deficiency

Following the above recommendations, a foliar Se fertilizer application dose of 20 g ha^{-1} on maize crops was used in Kiaga region. The cluster-randomized controlled trial found that, compared to the control, Se fertilization had a positive and significant effect on the Se concentration in maize grains of 0.448 mg kg^{-1}, corresponding to a relative difference of 94%. This was as a result of an increase in Se concentrations in biofortified maize grains by 0.391 mg kg^{-1}, which corresponds to a Se concentration increase of 0.02 mg kg^{-1} in maize grains, per 1 g of foliar sodium selenate fertilization of maize crops. The results are in line with 0.393 mg kg^{-1} (0.02 mg kg^{-1} per 1.0 g Se ha^{-1}) obtained during previous biofortification experiments based on the same application dose and technique in the same region (Chapter 4). These results are also in line with the effect of foliar Se fertilization on maize crops reported in literature for other countries, including 0.02 mg kg^{-1} per 1.0 g Se ha^{-1} in Malawi (Chilimba et al. 2012) and 0.01 mg kg^{-1} per 1.0 g Se ha^{-1} in China (Wang et al. 2017).

The thesis further evaluated dietary Se intake from maize based on the average daily amount of maize consumed by children and women at baseline and post-trial. Compared to the control group, the dietary Se intake from maize increased on average by 5.95 µg d^{-1}, corresponding to a relative difference of 103% for the children and by 16.35 µg d^{-1}, corresponding to a relative difference of 94% for the women. Notably, these Se intake results from the biofortified maize grains actually compare to half the Se intake from fish observed in the Lake Basin. This means that in favourable weather conditions in the Central Highlands whereby both maize and bean crops are biofortified, the Se biofortification strategy could be as effective as introducing an adequate fish intake in the Central Highlands.

When Se intake from biofortified maize grains is combined with Se intake from other foods consumed, the estimated effect of the biofortification intervention on the daily average dietary Se intake was equal to 10.95 µg day^{-1}, corresponding to a relative difference of 96% for children and 21.17 µg day^{-1} corresponding to a relative difference of 108% for women. The actual post-trial average dietary Se intake for the intervention group was equal to 20.25 µg day^{-1} for children and 37.47 µg day^{-1} for women, which is adequate among the children but 10.24 µg day^{-1} below the

adequate intake levels for women. This intervention would result in Se intake by children similar to that in countries with adequate Se intake such as 22.90 µg day^{-1} in Philippines (WHO 1989) and 19.30 µg day^{-1} in Germany (Lombeck et al. 1978). The resulting Se intake by women on the other hand would be similar to estimated dietary Se intake in European countries ranging between 30 and 50 µg day^{-1} (Fairweather-Taint et al. 2011). Generally, the biofortification intervention improved the average dietary Se intake by 46% and 44% among children and women, respectively. Comparing the post-trial average dietary Se intakes with respective EARs, the biofortification intervention decreased the risk of dietary Se deficiency by 44% for the children and 22% for the women. Due to limited food resources in rural areas, children's diet is given priority in the households during low food seasons, which explains the lower risk of dietary Se deficiency among the children at baseline, and the higher decrease in the risk of dietary Se deficiency for the children at post-trial. Generally, since there were no significant differences in observable factors of interest between the control and the treatment groups, and between the two phases of the experiment, the decrease in risk of dietary Se deficiency is considered to be a result of only the Se biofortification intervention.

7.3.3. Scoping implementation of Se biofortification strategy

The feasibility of implementation of Se biofortification needs to be considered. Economically, the cost of sodium selenate application varies mainly with the application dose. For instance, based on the current intervention trial and the local prices in the year 2017, a foliar application dose of 20 g Se ha^{-1} would cost 13 US$ per hectare applied as sodium selenate and 10 US$ per hectare if applied as sodium selenite. Therefore, an application rate of 10 g Se ha^{-1} for both maize and beans/other legumes, which is the typical scenario (maize and legumes are usually intercropped), would cost about 7 US$ for sodium selenate and 4 US$ for sodium selenite per hectare. Relying on information provision to farmers for them to increase dietary Se intake through agronomic biofortification will face various challenges as subsistence farmers without sufficient resources are unlikely to use micronutrient fertilizers. Therefore, in the absence of external incentives, only a few farmers would probably have the means to adopt such a strategy in their farm. For the majority of subsidence farmers, economic incentives combined with both agronomic and nutrition education would be necessary, at least in the short term, to foster biofortification as part of farm management practices. In any case, cost effectiveness of the strategy can be optimized through targeting the soil locations for which Se deficiency is high. Those are places where intervention would be the most beneficial for the affected communities. Acceptance of such intervention by farmers may also be influenced by the cultural and social background. Prevention of post-harvest losses is also prerequisite for an effective agronomic biofortification intervention, by ensuring that the rural communities are able to continue consuming the biofortified grains, and hence benefit from such an intervention.

In general, less costly and complementary long-term measures would need to be explored along the way. Yet, increasing the awareness of the public on the issue of Se deficiency and its diverse effects on human health will facilitate such a debate. The results and data presented by this thesis can form the basis for formulating incentives to promote use of micronutrient fertilizers among small-scale or subsistence farmers.

7.4. Limitations of the thesis and future research needs

7.4.1. Experimental design

The experiments presented in this thesis have limitations linked to some of the difficulties undergone during their implementation. First, difficult climate conditions (drought) experienced in most of the study areas resulted to crop failure, which restricted biofortification trials in Se deficiency locations of the highest priority. In addition, the drought affected the intervention trial whereby four out of the ten villages in Kiaga area were exempted from participation due to crop failure, leaving only six villages for the trials. In addition, the trial was not blinded and treatment allocation was not concealed. The required 5 clusters for the intervention were therefore reduced to three clustered per treatment group. In addition, crop failure decreased the options of target staple crops for the biofortification intervention. In terms of regional coverage, more study locations should be studied in the Lake Basin in order to provide a more representative data for the region. Second, there is need for further assessment of Se status of the target population using more sensitive biomarkers like blood i.e. selenoprotein P (SePP), and further investigate the impact of Se deficiency on health. Third, cultural beliefs and superstition were a major hindrance in most of the study locations. Indeed, sample data collection such as hair sampling for the Se status assessment survey was prevented by some of the communities that tended to associate biological samples such as blood, hair and nails with sorcery. Thus, conducting the research in some regions was particularly delicate. The research team was in several occasions forced to discontinue the research in some locations for safety reasons. Fourth, dietary intake assessment was conducted in only one season. Repeating the assessment in different seasons and under varying weather conditions would aid in capturing the seasonal variation of food availability and dietary intake. Fifth, all the dietary information from 24HR recalls depends on the respondent's memory and the skills of a well-trained interviewer. Exploring other methods for recording dietary intake would be valuable. Sixth and last, during the survey and at baseline for the biofortification intervention trial, selected households had in most cases already harvested their crops. This mixture of foodstuffs from farms and market may partly explain the observed lack of significant relationship between Se concentration in foods and soil characteristics of individual farms. Although climate conditions would also need to be considered, it could be relevant to conduct a study ensuring that only crops grown in the farm are sampled. Since the present research was primarily interested in

understanding Se supply in the diet, the analysis of all types of food consumed by the population irrespective of the source was however necessary.

7.4.2. Generalizability of findings and need for further investigation

One of the main criticisms related to the use of experiments concerns the generalizability or external validity of the results and their representativeness to the overall population/farmlands concerned by the issue. The intervention trial was conducted in only one study location under similar agricultural soil type and climatic conditions. While this influence is also to be expected in different study locations and different soil conditions, there is a need for further biofortification intervention trials in other study locations with different agricultural soils and climatic conditions, and especially in the Mbuyu area which was of the highest priority. A second factor that needs to be considered in regard to the generalizability of the results, is the conversion of Se content in consumed foodstuffs into Se supply for the body. This assumption lies on the fact that over 80% of dietary Se is absorbed in the body. More accurate information on the Se bioavailability of various foods would however be necessary for a better assessment of dietary Se intake. In addition, more sensitive Se status measurement through SePP in the blood of the target population would be preferred in future studies. Installation of analytical capacity (e.g. ICP-MS) in Kenya or surrounding countries would also be useful in order to make Se analysis locally possible, and to cut on the expensive sample shipment costs especially in the case of biological samples.

7.4.3. Variability of Se concentration in foodstuffs and intakes

This section raises the potential issue related to variability of Se concentration in foodstuffs for control groups over the 3 years of the thesis research. For instance, the mean Se concentration in maize grains in the Kiaga location was equal to 0.158 (SD 0.006) during the Se status survey in August 2015, 0.048 (SD 0.008) for the biofortification trials in August 2016, and 0.106 (SD 0.099) for the intervention trial in April and September 2017. Mean comparison tests show no significant difference of Se concentrations across these years at 95% confidence level (between the year 2015 and 2016: p-value = 0.051; between 2015 and 2017: p-value = 0.145, and between 2016 and 2017: p-value = 0.420). Similarly, the mean maize Se concentration does not vary significantly in control groups between 2015 and 2016, for both Mbeu (p-value = 0.050) and Mbuyu (p-value = 0.052). Yet, one of the shortcoming for this comparison is the large difference in sample size across the years. The number of maize samples (each representing a farm) across the three years for Kiaga were equal to 9, 2, and 31. In for Mbeu and Mbuyu, each locations had 19 and 4 maize samples (each representing a farm) in 2015 and 2016, respectively. Because of the sample size and because differ farms/households were sampled in each of the years, it is very difficult to conclude on what could appear as “variability” of Se concentrations in the control, based on the comparison of means and their standard deviations.

Various factors could also explain such potential variability. First, the farms involved in the biofortification trials were selected based on the findings of Se concentration in the soil and foods resulting from the 2015 assessment survey. Mbuyu was identified as the most Se deficient location. Farms with similar low Se concentration in soil and foods from Mbeu and Kiaga were selected for the biofortification trials. This provided a common basis for comparing crop's response to Se fertilization across the three study locations. The selected farms for biofortification experiments were therefore lower in Se status compared to the means reported by the assessment survey. Second, there is no standard agricultural practice for the subsistence farming within a study location nor between the study locations. Some farmers apply fertilizers with no consistent pattern, others apply animal manure occasionally, while other farmers do not undertake any action for replenishing depleted soil's nutrients. Third, over the last decades, climate change has resulted in important changes in seasonal weather patterns, which was in fact also observed during this time period of the research. Most of the study locations experienced adequate rainfall and good crops' growth and harvest in 2015. During the biofortification trials in 2016, Mbuyu and Mbue experienced moderate rainfall, while Kiaga suffered a drought which resulted in crop's failure. Most of the farmers in Kiaga had little or no maize harvest. The soil biofortification experiment was particularly affected in Kiaga due to lack of early rains, resulting in crop failure especially for beans. In 2017, the climatic conditions reversed between Kiaga and Mbuyu: Mbuyu suffered a severe drought that wiped over 70% of crops. Although Mbuyu had the highest risk of dietary Se deficiency, it was not possible to conduct a biofortification intervention trail in this location. Kiaga, which had received some early rains was selected instead. However, due to the failure of the second rains, the foliar Se applications for the biofortification intervention was conducted in a dry season contrary to the recommendations for application during the rainy season, on a sunny day. Due to the dependence on rain-fed agriculture, the fluctuations in rainfall between the study periods of 2015 to 2017 contributed to variation in both local the food production and food species available.

These factors potentially affect Se uptake by crops either via the roots or foliar application. The thesis therefore recommends further research that accounts for these factors. This includes ideally biofortification experiments using farms having common agricultural practices. In addition, experiments and intervention trials should be repeated for several years in the same study location(s) in order to account for the seasonal or annual variability in climatic conditions. The coverage of both the Se status survey and biofortification trials in Kenya should also be extended to other regions/soil types in order to provide a representative national Se deficiency risk mapping.

7.4.4. Cost effectiveness: dietary diversification versus Se biofortification

Further research is needed to evaluate the cost of reaching the international standards of diet quality in a setting like the one studied in this thesis. This includes the cost of diet diversity for including at least five food groups as defined by the widely used minimum dietary diversity for

women (MDD-W) indicator (Martin-Prevel 2017), or at least four food groups for children as defined by infant and young child minimum dietary diversity indicator. These costs can then be compared to a cost of Se intake adequacy indicator for the lowest-cost way to meet Se EARs. Such information would help indicate how affordable a nutritious diet is, and which nutritional criteria account for the change. As explained by Masters et al. (2019), necessary secondary data include the national average monthly food prices from sources such as the agricultural market information system and national consumer price indexes. Moreover, according to such study by Save the Children (2017) in North-West Kenya, primary data can also be corrected through market surveys, village interviews, and focus group discussions, aimed at collecting data on market prices, seasonal availability, and dietary habits for all available foods. Notably, prices for staple foods are among the highest in the Eastern and Southern Africa region (Ariga et al. 2010). For instance, Save the Children (2017) found that between 2,150.00 to 2,850.00 euros are needed annually per household in order to close the affordability gap and enable households to purchase a nutritious diet. However it was noted that these results were execrated by the current effect of climate change which has resulted in a widespread drought. Generally, it is expected that the cost for closing the affordability gap for a nutritious diet in other more agricultural productive regions such as the Central Highlands and the Lake Basin would be lower compared to North-West Kenya. In this regard, further analysis of the cost of diet diversity needs to be extended to other regions in Kenya.

In order to compare the cost effectiveness of the two strategies, the cost of diet diversity needs to be compared to the cost of Se biofortification strategy for a same increase of Se dietary intake in the population. In a broad sense, considering that Se fertilization on crops is required only once per year or twice for regions with two harvest seasons, it would cost between 11 to 30 euro/year/acres to achieve adequate dietary Se intake through Se fertilization, depending on whether only maize crops are biofortified or both maize and legume crops. Thus, Se biofortification would be by far cheaper than achieving a diversified diet for a defined adequate dietary Se intake. The surplus of the biofortified grains can then be supplied to more arid areas such as Northern Kenya. In general, population-based approaches involving food biofortification appear to be more cost effective (Tulchinsky 2010). Generally, as recommended by Townsend (2015), a combined solution is needed that includes biofortification, diet diversity and other strategies in order to improve nutritional outcomes.

7.4.5. Advocacy for agronomic biofortification

The findings of this thesis call for the need to address Se deficiency through a combination of agricultural- and nutritional-focused campaigns and policy measures. Besides Se deficiency, Kenya faces a high prevalence of micronutrient deficiencies of which biofortification offers a dietary agriculture-based solution. Future policy research needs to focus on the advocacy for agronomic biofortification. Without adequate policies for biofortification strategies, it will be difficult to

implement such potential solutions to tackle micronutrient deficiencies. Future research should therefore mix the evidence generated from this thesis that matched Se biofortification strategy to the local context, with promotion to change the attitudes of the rural population (subsistence farmers) among other stakeholders, and the development of national standards for agronomic biofortification. This will form a basis to advocate for national biofortification policies. The understanding of the multiple factors contributing to Se deficiency within the food system is vital in targeting and prioritizing among the possible policy instruments.

A wide range of national and international public officials recognize the significant impact of biofortification for improving and sustaining public health. Significant progress has already been made in integrating biofortification into regional and national policies in some African countries. For instance, Malawi and Uganda have implemented biofortification in their national strategies, with the goal to end malnutrition by 2025, and countries such as Rwanda and Zambia have included biofortified crops in their national agriculture and nutrition plans (Bouis and Saltzman 2017). Since the Kenya national food security and nutrition policy already includes food fortification (Ministry of Health Kenya, 2013, Fiedler et al. 2014), future policy research should rather focus on the inclusion of agronomic biofortified foods. Besides, fortification of processed food with Se has also been reported in table salt (Ning et al. 2015).

In addition to development and adoption of Se biofortified foods, policy research also needs to focus on increased nutrition education through agricultural extension and livelihoods programs that can improve dietary variety in production and increase dietary Se intake. In addition, farmers' incentives should be aligned through associated policies to allow farmers to respond to changing market demands. Furthermore, agricultural support should be aligned to the adoption of more climate-resistant practices and technologies.

REFERENCES

Alfthan, G. and Neve, J. 1996. Reference values for serum selenium in various areas evaluated according to the TRACY protocol. *Journal of Trace Elements in Medicine and Biology*, 10: 77-87

Amare, B., Moges, B., Fantahun, B., Tafess, K, Woldeyohannes, D., Yismaw, G., Ayane, T., Yabutani, T., Mulu, A., Ota, F., Kassu, A. 2012. Micronutrient levels and nutritional status of school children living in Northwest Ethiopia. *Nutrition Journal*, 11:108

Ariga, J., Jayne, TS., Njukia, S. 2010. Staple food prices in Kenya. Comesa-MSU-IFPRI African Agricultural Marketing Project (AAMP). https://core.ac.uk/download/pdf/6678899.pdf. Accessed on 4 May 2019

Arimond, M., Wiesmann, D., Becquey, E., Carriquiry, A., Daniels, MC., Deitchler, M., Fanou-Fogny, N., Joseph, M. L., Kennedy, G., Martin-Prevel, Y. 2010. Simple Food Group Diversity Indicators Predict Micronutrient Adequacy of Women's Diets in 5 Diverse, Resource-Poor Settings. *The Journal of Nutrition*, 140: 2059-2069

Aro, A., Alfthan, G., Varo, P. 1995. Effects of supplementation of fertilizers on human selenium status in Finland. *Analyst*, 120(3): 841-3

Arthur, JR., Beckett, GJ., Mitchell, JH. 1999. The interactions between selenium and iodine deficiencies in man and animals. *Nutrition Research Reviews*, 12: 55-73

Arvy, MP. 1993. Selenate and selenite uptake and translocation in bean plants (Phaseolus vulgaris). *Journal of Experimental Botany*, 44: 1083-87

Ashour, MN., Salem, SI., El-Gadban, HM., Elwan, N, Basu, TK. 1999. Antioxidant status in children with protein-energy malnutrition (PEM) living in Cairo, Egypt. *European Journal of Clinical Nutrition*, 52: 669-673

Ashton, K., Hooper, L., Harvey, LJ., Hurst, R., Casgrain, A., Fairweather-Tait, SJ. 2009. Methods of assessment of selenium status in humans: a systematic review. *American Journal of Clinical Nutrition*, 89(6): 2025-2039

Aslam, M., Harbit, KB., Huffaker, RC. 1990. Comparative effects of selenite and selenate on nitrate assimilation in barley seedlings. *Plant Cell Environment*. 13:773-82

Austin, S. and Soloway, N. 2012. Hair Mineral Analysis. *Textbook of Natural Medicine*, 150-156.

Axelsson, MD., Olofsson, R., Environment, T. 2001. Reference data Trace elements in human biological material. ALS Scandinavia. https://www.alsglobal.se/media-se/pdf/reference_data_biomonitoring_120710.pdf. Accessed on 15 July 2018

Baeten, JM., Mostad, SB., Hughes, M., Overbaugh, J., Bankson, DD., Mandaliya, K., Ndinya-Achola, JO., Bwayo, JJ., Kreiss, JK. 2001. Selenium deficiency is associated with shedding of HIV-1 infected cells in the female genital tract. *Journal of Acquired Immune Deficiency Syndromes*, 26: 360 - 364

Bain LE., Awah, PK., Geraldine, N., Kindong, NP., Sigal, Y., Bernard. N., Tanjeko, AT. 2013. Malnutrition in Sub – Saharan Africa: burden, causes and prospects. *Pan African Medical Journal*, 15: 120-2535

Balirwa, JS., Chapman, CA., Chapman, LJ., Cowx, IG., Geheb, K., Kaufman, L. 2003. Biodiversity and fisheries sustainability in the Lake Victoria Basin: an unexpected marriage? *Bioscience*, 53: 703-16

Batzevich, VA. 1995. Hair trace element analysis in human ecology studies. *The Science of the Total Environment*, 164: 89-98

Beauchemin, S. and Simard, RR. 1999. Soil phosphorus saturation degree: Review of some indices and their suitability for P management in Québec, Canada. *Canadian Journal of Soil Science*, 79(4), 615–625

Biesalski, HK. 2013. Hidden hunger. Springer & Business Media, ISBN 978-3-642-33949-3

Biri, A., Bozkurt, N., Turp, A., Kavutcu, M., Himmetoglu, O., Durak, I. 2007. Role of oxidative stress in intrauterine growth restriction. *Gynecologic and Obstetric Investigation*, 64(4): 187-192

Blaylock, MJ. and James, BR. 1994. Redox transformations and plant uptake of selenium resulting from root-soil interactions. *Plant and Soil*, 158(1): 1-12

Blazina, T., Sun, Y., Voegelin, A., Lenz, M., Berg, M., Winkel, L.H.E. 2014. Terrestrial selenium distribution in China

ispotentially linked to monsoonal climate. *Nature Communication*, DOI: 10.1038/ncomms5717

Bouis, HE. and Saltzman, A. 2017. Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Global Food Security,* 12: 49-58

Bouis, HE. and Welch, RM. 2010. Biofortification - A Sustainable Agricultural Strategy for Reducing Micronutrient Malnutrition in the Global South. *Crop Science,* 50, S20-S32

Bouis, HE., Hotz, C., McClafferty, B., Meenakshi, JV., Pfeiffer, WH. 2011. Biofortification: a new tool to reduce micronutrient malnutrition. *Food and nutrition bulletin,* 32, S31-S40

Bratakos, MS., Zafiropoulos, TF., Sislos, PA., Ioannou, PV. 1988. Selenium losses on cooking Greek foods. *International Journal of Food Science and Technology*, 23: 585-590

Brigelius-Flohe,´ R. and Maiorino, M. 2013. Glutathione peroxidases. *Biochimica et Biophysica Acta*, 1830: 3289-3303

Broadley, MR., Alcock, J., Alford, J., Cartwright, P., Foot, I., Fairweather-Tait, SJ., Zhao, FJ. 2010. Selenium biofortification of high-yielding winter wheat (Triticum aestivum L.) by liquid or granular Se fertilization. *Plant and Soil*, 33: 5-18

Broadley, MR., White, PJ., Bryson RJ., Meacham, MC., Bowen, HC., Johnson, SE., Hawkesford, MJ., McGrath, SP., Zhao, FJ., Breward, N., Harriman, M., Tucker, M. 2006. Biofortification of UK food crops with selenium. *Proceedings of the Nutrition Society,* 65: 169-181

Broome, CS., McArdle, F., Kyle, JA. 2004. An increase in selenium intake improves immune function and poliovirus handling in adults with marginal selenium status. *American Journal of Clinical Nutrition*, 80: 154-62

Brouwer, W., Kroeze, W., Crutzen, R., de Nooijer, J., de Vries, NK., Brug, J., Oenema, A. 2011. Which intervention characteristics are related to more exposure to internet-delivered healthy lifestyle promotion interventions? A systematic review. *Journal of Medical Internet Research*, 13: 25-43

Bueno, M., Pannier, F., Potin-Gautie, M. 2007. Determination of Organic and Inorganic Selenium Species Using HPLC-ICP-MS. Agilent Technologies, Inc, 5989-7073

Burk, RF., Hill, KE. 2009. Selenoprotein P-Expression, functions and roles in mammals. *Biochemica et Biophysica Acta*, 1790: 1441-1447

Bwibo, NO. and Neumann, CG. 2003. The Need for Animal Source Foods by Kenyan Children. *The Journal of Nutrition*, 133: 3936-3940

Cakmak, I. 2014. Agronomic biofortification. Conference brief 8, In: Proceedings of the 2nd Global Conference on Biofortification: Getting Nutritious Foods to People, Rwanda

Cakmak, I., Kalaycı, M., Ekiz, H., Braun, HJ., Kılınç, Y., Yılmaz, A. 1999. Zinc deficiency as a practical problem in plant and human nutrition in Turkey: a NATO-science for stability project. *Field Crops Research*, 60: 175-88

Carter, MR. and Gregorich, EG. 2007. Soil Sampling and Methods of Analysis (2nd edition). CRC Press. Canadian Society of Soil Science

Chege, PM. and Ndungu, ZW. 2016. Opportunities and Limitations of Using Food Composition Tables in Clinical Nutrition and Dietetics in Kenya. EC *Nutrition* 6.1: 24-27

Chen, L., Yang, F., Xu, J., Hu, Y., Hu, Q., Zhang, Y., Pan G. 2002. Determination of selenium concentration of rice in China and effect of fertilization of selenite and selenate on selenium content of rice. *Journal of Agricultural and Food Chemistry*, 50: 5128-5130

Cheserek, MJ., Waudo, JN., Tuitoek, PJ., Msuya, JM., Kikafunda, JK. 2012. Nutritional vulnerability of older persons living in urban areas of Lake Victoria Basin in East Africa: A Cross Sectional Survey. Journal of *Nutrition in Gerontology and Geriatrics*, 31: 86-96

Chilimba, AD., Young, SD., Black, CR., Rogerson, KB., Ander, EL., Watts, MJ. Lammel, J., Broadley, MR. 2011. Maize grain and soil surveys reveal suboptimal dietary selenium intake is widespread in Malawi. *Scientific Reports*, 1: 72

Chilimba, ADC., Young, SD., Black, CR., Meacham MC., Lammel J., Broadley MR. 2012. Agronomic biofortification of maize with Selenium (Se) in Malawi. *Field Crops Research,* 125: 118-128

Clark, LC., Combs, GF., Turnbull, BW., Slate, EH., Chalker, DK., Chow, J. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. *Nutritional prevention of cancer study group,* 276: 1957-63

Clausen, J., Nielsen, SA. 1998. Comparison of whole blood selenium values and erythrocyte glutathione peroxidase activities of normal individuals on supplementation with selenite , selenite, L-selenomethionine, and high selenium yeast. *Biological Trace Element Research*, 15: 125-138

Combs, GF., Watts, JC., Jackson, M., Johnson, LK., Zeng, H., Scheet, AJ., Uthus, EJ., Schomburg, L., Hoeg, A., Hoefig, CA. 2011. Determinants of selenium status in healthy adults. *Nutrition Journal*, 10: 75–82

Combs, GF. 2001. Selenium in global food systems. *British Journal of Nutrition,* 85(05), 517

Combs, GF. 2015. Biomarkers of selenium status. *Nutrients*, 7: 2209-2236

Council National Research (US). 1984. National Survey Data on Food Consumption: Uses and Recommendations. Washington, DC: The National Academies Press. https://doi.org/10.17226/733

Cuvardic, MS. 2003. Selenium in the soil. *Proceedings for Natural Sciences*, 104: 23-37 UDC 631.416:546.23

Dapi, LN., Omoloko, C., Janlert, U., Dahlgren, L., Håglin, L. 2007. I eat to be Happy, to Be Strong, and to Live. Perceptions of Rural and Urban Adolescents in Cameroon, Africa. *Journal of Nutrition Education and Behaviour*, 39: 320-326

Darnton-Hill, I., Webb, P., Harvey, PW., Hunt, JM., Dalmiya, N., Chopra, M., Ball, MJ., Bloem, MW., de Benoist. B. 2005. Micronutrient deficiencies and gender: social and economic costs. *The American Journal of Clinical Nutrition*, 81(5): 1198-1205

De Temmerman, L., Waegeneers, N., Thiry, C., Du Laing, G., Tack, F., Ruttens, A. 2014. Selenium content of Belgian cultivated soils and its uptake by field crops and vegetables. *Science of the Total Environment*, 468-469: 77-82

Dhillon, KS. and Dhillon, SK. 1999. Adsorption–desorption reactions of selenium in some soils of India. *Geoderma*, 93: 19-31

Dhillon, SK. and Dhillon, KS. 2000. Selenium adsorption in soils as influenced by different anions. *Journal of Plant Nutrition and Soil Science*, 163(6): 577-582

Dijkshoorn, K., Macharia, P., Huting, J., Maingi, P., Njoroge, C. 2011. Soil and Terrain conditions for the Upper Tana River catchment, Kenya (ver1.1). Green Water Credits Report 11 / ISRIC Report 2010/09b. http://www.isric.org/isric/Webdocs/Docs/ISRIC_Report_2010_09b.pdf. Accessed on 11 August 2018

Diriba, BK., Joy, EJM., Young, SD., Odee, DW., Ander, EL., Broadley, MR. 2017a. Variation in the mineral element concentration of *Moringa oleifera* Lam. and *M. stenopetala* (Bak.f.) Cuf.: Role in human nutrition. *PLOS ONE*, Doi.org/10.1371/journal.pone.0175503

Diriba, BK., Joy, EJM., Young, SD., Odee, DW., Ander, EL., Magare, C., Gitu, J., Broadley, MR. 2017b. Challenges and opportunities for Moringa growers in southern Ethiopia and Kenya. PLOS ONE, Doi.org/10.1371/journal.pone.0187651

Drain, PK., Baeten, JM., Overbaugh, J. 2006. Low serum albumin and the acute phase response predict low serum selenium in HIV-1 infected women. *BMC Infectious Diseases*, 6: 85

Du Laing, G. 2010. Analysis and Fractionation of trace element in Soils. In: Hooda PS, editor. Trace Elements in Soils, 53-80. Chichester, UK: Wiley-Blackwell

Du Laing, G., Rinklebe, J., Vandecasteele, B., Meers, E., Tack, FMG. 2009. Trace metal behaviour in estuarine and riverine floodplain soils and sediments: A review. *Science of the total environment*, 407: 3972-3985

Ducsay, L., Lozek, O., Marcek, M., Varenyiova, M., Hozlar, P., Losak, T. 2016. Possibility of selenium biofortification of winter wheat grain. *Plant Soil Environment*, 62, 8: 379-383

Dudek, JA., Elkins, ER., Behl, BA. 1989. Effect of cooking and canning on the mineral content of selected sea foods. *Journal of Food Composition and Analysis*, 2: 273-285

Dumont, E., Vanhaecke, F., Cornelis, R. 2006. Selenium speciation from food source to metabolites: A critical review. *Analytical and Bioanalytical Chemistry*, 385: 1304-1323

Eich-Greatorex, S., Krogstad, T., Sogn, TA. 2010. Effect of phosphorus status of the soil on selenium availability. *Journal of Plant Nutrition and Soil Science*, 173(3): 337-344

Ekholm, P., Reinivuo, H., Mattila, P., Pakkala, H., Koponen, J., Happonen, A., Hellstro¨m, J., Ovaskainen, M-J. 2007. Changes in the mineral and trace element contents of cereals, fruits, and vegetables in Finland. *Journal of Food Composition and Analysis*, 20: 487-495

El Mehdawi, AF., Quinn, CF., Pilon-Smits, EA. 2011. Selenium hyperaccumulators facilitate seleniumtolerant neighbors via phytoenrichment and reduced herbivory. *Current Biology*, 21: 1440-1449

Fairweather-Tait, SJ., Bao, Y., Broadley, MR., Collings, R., Ford, D., Hesketh, JE., Hurst, R. 2011. Selenium in Human Health and Disease. *Antioxidants and Redox Signaling*, 14(7): 1337-1383

Fairweather-Tait, SJ., Collings, R., Hurst, R. 2010. Selenium bioavailability: Current knowledge and future research requirements. *American Journal of Clinical Nutrition*, 91: 1484-1491

Fanzo, J. 2012. The Nutrition Challenge in Sub-Saharan Africa. United Nations Development Program. Regional Bureau for Africa, WP 012

Fanzo, J., Hunter, D., Borelli, T., Mattei, F. 2012. Diversifying food and diets: Using agricultural biodiversity to improve nutrition and health. *Biodiversity International*. ISBN: 978-0-203-12726

FAO (Food and Agriculture Organization), IFAD (International Fund for Agricultural Development), UNICEF (United Nations Children's Fund), WFP (World Food Programme), WHO (World Health Organization). 2018. The State of Food Security and Nutrition in the World 2018. Building climate resilience for food security and nutrition. Rome, FAO. Licence: CC BY-NC-SA 3.0 IGO

FAO (Food and Agriculture Organization). 2000. The State of Food Insecurity in the World. Rome: FAO; FAO/21846/R

FAO (Food and Agriculture Organization). 2009. World Food Summit: Rome Declaration and Plan of Action. FAO; November 2009. http://www.fao.org/fileadmin/templates/wsfs/Summit/Docs/Declaration/WSFS09_Draft_Declaration.pdf. Accessed on 4 April 2019

FAO (Food and Agriculture Organization). 2016. Climate change and food security: risks and responses. ISBN 978-92-5-108998-9

FAO/WHO (Food and Agriculture Organization/Word Health Organization). 2004. Vitamin and mineral requirements in Human Nutrition. Rome: FAO. ISBN 92-4-154612-3

Ferretti, RJ. and Levander, OA. 1974. Effect of milling and processing on the selenium content of grains and cereal products. *Journal of Agriculture and Food Chemistry*, 22: 1049-1051

Fiedler, J., D'Agostino, A., Sununtnasuk, C. 2014. A Rapid Initial Assessment of the Distribution and Consumption of Iron-Folic Acid Tablets through Antenatal Care in Kenya. Nutrition Technical Brief. Arlington, VA: USAID/Strengthening Partnerships, Results and Innovations in Nutrition Globally (SPRING) Project

Fiorella, KJ., Hickey, MD., Salman, CR., Nagata, JM., Mattah, B., Magerenge, R., Cohen, CR., Bukusi, EA., Brashares, JS., Fernald, LH. 2014. Fishing for food? Analyzing links between fishing livelihoods and food security around Lake Victoria, Kenya. *Food Security*, 6: 851-860

Forber, KJ., Withers, PJA., Ockenden MC., Haygarth PM. 2018. The Phosphorus Transfer Continuum: A Framework for Exploring Effects of Climate Change. *Agricultural and Environmental Letters*, 3: 180036

Fordyce, F. 2007. Selenium geochemistry and health. *A Journal of the Human Environment*, 36: 94-97

Fordyce, F M., Brereton, N., Hughes, J., Luo, W., Lewis, J. 2010. An Initial Study to Assess the Use of Geological

Parent Materials to Predict the Se Concentration in Overlying Soils and in Five Staple Foodstuffs Produced on Them in Scotland. *Science of the Total Environment*, 408: 5295-5305

Fordyce, FM. 2013. Selenium deficiency and toxicity in the environment. In Essentials of Medical Geology: Revised Edition, pp. 375-416. Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-007-4375-5_16

Foster, HD. 2003. Why HIV-1 has diffused so much more rapidly in sub-Saharan Africa than in North America. *Medical Hypotheses*, 60(4): 611-614

Furman, C., Rundlof, AK., Larigauderie, G., Jaye, M., Bricca, G., Copin, C. 2004. Thioredoxin reductase 1 is upregulated in atherosclerotic plaques: specific induction of the promoter in human macrophages by oxidized low-density lipoproteins. *Free Radical Biology & Medicine*, 37: 71-85

Gachene, CKK. and Kimaru, G. 2003. Soil Fertility and Land Productivity - A guide for extension workers in the eastern Africa region. Technical Handbook No.30. Regional Land Management Unit (RELMA)/ Swedish International Development Cooperation Agency Sida, 9966-896-66-X

Gao, Y., Duan, A., Qiu, X., Liu, Z., Sun, Zhang, J., Wang, H. 2010. Distribution of roots and root length density in a maize/soybean strip intercropping system. *Agricultural Water Management*, 98: 199-212

Garg, M., Sharma, N., Sharma, S., Kapoor, P., Kumar, A., Chunduri, V., Arora, P. 2018. Biofortified Crops Generated by Breeding, Agronomy, and Transgenic Approaches Are Improving Lives of Millions of People around the World. *Frontiers in Nutrition*, 5: 12

Gashu, D., Stoecker, BJ., Bougma, K., Adish, A., Haki, GD., Marquis, GS. 2016. Stunting, selenium deficiency and anemia are associated with poor cognitive performance in preschool children from rural Ethiopia. *Nutrition journal*, 15: 38

Ge, K. and Yang, G. 1993. The epidemiology study of endemic of selenium deficiency diseases in the etiological study of endemic disease in China. *The American Journal of Clinical Nutrition*, 57(5): 259–263

Geheb, K., Kalloch, S., Medard, M., Nyapendi, A., Lwenya, C., Kyangwa, M. 2008. Nile perch and the hungry of Lake Victoria: gender, status and food in an East African fishery. *Food Policy*, 1: 85-98

Giachene, C. and Kimaru, G. 2003. *Soil fertility and land productivity*: A guide for extension workers in the eastern Africa region. Technical Handbook No. 30. http://www.worldagroforestry.org/downloads/Publications/PDFS/B13197.pdf. Accessed on 10 June 2018

Gibson, RS. and Hotz, C. 2002. Dietary diversification/modification strategies to enhance micronutrient content and bioavailability of diets in developing countries. *British Journal of Nutrition*, 85: 159-166

Gissel-Nielsen, G., Gupta, UC., Lamand, M., Westermarck, T. 1984. Selenium in soils and plants and its importance in livestock and human-nutrition. *Advances in Agronomy*, 37: 397−460

Goh KH. and Lim TT. 2004. Geochemistry of inorganic arsenic and selenium in a tropical soil: effect of reaction time, pH, and competitive anions on arsenic and selenium adsorption. *Chemosphere*, 55: 849-859

Graham, RD., Knez, M., Welch RM. 2012. How Much Nutritional Iron Deficiency in Humans Globally Is due to an Underlying Zinc Deficiency? *Advances in Agronomy*, 115: 1-40

Graham, RD., Welch, RM., Saunders, DA., Hyphen, TG., Bouis, HE., Bonierbale, M., de Haan, S., Burgos, G., Thiele, G., Liria, R., Meisner, CA., Beebe, SE., Potts, MJ., Kadian, M., Hobbs, PR., Gupta, RK., Twomlow, S. 2007. Nutritious Subsistence Food Systems, in: L.S.Donald (Ed.), *Advances in Agronomy*. Academic Press: 1-74

Gu, QP., Xia, XM., Ha, PC., Butler, JA., Whanger, PD. 1998. Distribution of selenium between plasma fractions in guinea pigs and humans with various intakes of selenium. *Journal of Trace Elements in Medicine and Biology*, 12: 8-15

Gupta, M. and Gupta, S. 2017. An Overview of Selenium Uptake, Metabolism, and Toxicity in Plants. *Frontiers in Plant Science*, 7

Gupta, S., Venkatesh, A., Ray, S., Srivastava, S. 2014. Challenges and prospects for biomarker research: A current

perspective from the developing world. *Biochimica et Biophysica Acta*, 1844: 899-908

Gupta, UC. and Gupta, SC. 2002. Quality of animal and humanlife as affected by selenium management of soils and crops. *Communications in Soil Science and Plant Analysis* 33: 15-18

Hamilton, SJ. 2004. Review of selenium toxicity in the aquatic food chain. *Science of the Total Environment*, 326: 1-31

Harrison, GG. 2010. Public health interventions to combat micronutrient deficiencies. *Public Health Reviews*, 32: 256-266

Hart, DJ., Fairweather-Tait, SJ., Broadley, MR., Dickinson, SJ., Foot, I., Knott, P., McGrath, SP., Mowat, H., Norma, K, Scott, R., Stroud, JL., Tucker, M., White, PJ., Zhao, FJ., Hurst, R. 2011. Selenium concentration and speciation in biofortified flour and bread: Retention of selenium during grain biofortification, processing and production of Se-enriched food. *Food Chemistry*, 126: 1771-1778

Hatloy, A., Torheim, LE., Oshaug, A. 1998. Food Variety - A Good Indicator of Nutritional Adequacy of the Diet? A Case Study from an Urban Area in Mali, West Africa. *European Journal of Clinical Nutrition*, 52: 891-8

Haug, A., Graham, RD., Christophersen, OA., Lyons, GH. 2007. How to use the world's scarce selenium resources efficiently to increase the selenium concentration in food. *Microbial Ecology in Health and Disease*, 19: 209-228

Hayes, R. and Bennett, S. 1999. Simple sample size calculation for cluster-randomized trials. *International Journal of Epidemiology*, 28: 319-32

Higgs, DJ., Morris, VC., Levander, OA. 1972. Effect of cooking on selenium content of foods. *Journal of Agriculture and Food Chemistry*, 20: 3

Hodges, R., Bernard, M., Rembold, F. 2014. APHLIS - Postharvest cereal losses in Sub-Saharan Africa, their estimation, assessment and reduction. European Union, 978-92-79-43852-3

Hurst, R., Armah, CH., Dainty, JR., Dave, JH., Teucher, B., Goldson, AJ., Broadley, MR., Motley, AK., Fairweather-Tait, SJ. 2010. Establishing optimal selenium status: results of a randomized, double-blind, placebo-controlled trial. *The American Journal of Clinical Nutrition*, 91: 923-931

Hurst, R., Siyame, EWP., Young, SD., Chilimba, ADC., Joy, EJM., Black, CR., Ander, WL., Watts, MJ., Chilima, B., Gondwe, J., Kang'ombe, D., Stein, A.J., Fairweather-Tait, SJ., Gibson, RS., Kalimbira, AA., Broadley, MR. 2013. Soil-type influences human selenium status and underlies widespread selenium deficiency risks in Malawi. Scientific Reports, 3(2): 1-6

IAEA (International Atomic Energy Agency). 1994. Application of Hair As an Indicator for Trace Element Exposure in Man - A review. https://inis.iaea.org/collection/NCLCollectionStore/_Public/26/032/26032943.pdf?r=1&r=1. Accessed on 3 May 2018

Institute of Agricultural Policy, T. 2013. Kenya Country Survey Report on FTF Zone of Influence Population Based Survey. https://www.feedthefuture.gov/wp-content/uploads/2018/03/Kenya_ZOI_Baseline_Report_May2013.pdf. Accessed on 1 September 2018

Institute of Medicine. 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: The National Academies Press. https://doi.org/10.17226/9810

International Livestock Research Institute. 2015. Feed the Future Kenya Accelerated Value Chain Development Program: Accelerating value chains development for smallholder farmers and pastoralists with technologies and innovations — Program document. https://cgspace.cgiar.org/bitstream/handle/10568/69538/avcd_program_report.pdf?sequence=5%0Ahttps://cgspace.cgiar.org/handle/10568/69538. Accessed on 20 August 2018

Jackson, MI., Lunoe, K., Gabel-Jensen, C., Gammelgaard, B., Combs, GF. 2013. Metabolism of selenite to selenosugar and trimethylselenonium in vivo: Tissue dependency and requirement for S-adenosylmethioine-

dependent methylation. *Journal of Nutritional Biochemistry*, 24: 2023-2030

Jacobs, L. W. (Ed.). 1989. Selenium in Agriculture and the Environment, *Soil Science Society of America Special Publication* 23, SSSA, Madison, WI.

Jaetzold, R., Schmidt, H., Hornetz, B., Shisanya, C. 2006. Farm Management Handbook of Kenya: Central Kenya (Rift Valley and Central Provinces). 2nd Edition. http://library.wur.nl/isric/fulltext/isricu_i00023897_001.pdf. Accessed on 20 October 2018

Jarvis, DI., Padoch, C., Cooper, D. 2006. Managing Biodiversity in Agricultural Ecosystems. New York: Columbia University Press, ISBN: 9778-0-231-1364-8

Jayne, TS. and Muyanga, M. 2012. Land constraints in Kenya's densely populated rural areas: implications for food policy and institutional reform. *Food Security*, 4: 399-421

Jezek, P., Škarpa, T., Losak, J., Hlusek, M., Elzner, J., Elzner, P. 2012. Selenium - An Important Antioxidant in Crops Biofortification, In: Antioxidant Enzyme, Mohammed Amr El-Missiry M.A. (Ed.), InTech, Rijeka, Croatia, 343-368.

Jiang, XM., Cao, XY., Jiang, JY., Ma, T., James, DW., Rakeman, MA., Duo, ZH., Mamette, M., Amette, K., Zhanq, ML, Delonq, GR., 1997. Dynamics of environmental supplementation of iodine: four years' experience in iodination of irrigation water in Hotien, Xinjiang, China. *Archives of Environmental Health*, 52: 399-408

Johns, T. and Eyzaguirre, P. 2006. Linking biodiversity, diet and health in policy and practice. *Proceedings of the Nutrition Society*, 65: 182-189.

Jones, GD., Droz, B., Greve, P., Gottschalk, P., Poffet, D., McGrath, SP., Seneviratne, SI., Smith, P., Winkel, LH. E. 2017. Selenium deficiency risk predicted to increase under future climate change. *PNAS* Early Edition

Joy, EJ., Broadley, MR., Young, SD., Black, CR., Chilimba, AD., Ander, EL., Barlow, TS., Watts, MJ. 2015. Soil type influences crop mineral composition in Malawi. *Science of the Total Environment*, 505: 587-95

Joy, EJM., Ander, EL., Young, SD., Black, CR., Watts, MJ., Chilimba, ADC., Broadley, MR. 2014. Dietary mineral supplies in Africa. *Physiologia Plantarum*, 151(3): 208-229

Kaminski, J. and Christiaensen, L. 2014. Post-Harvest Loss in Sub-Saharan Africa. The World Bank, WPS6831

Karmakar, R., Das, I., Dutta, D., Rakshit, A. 2016. Potential Effects of Climate Change on Soil Properties: A Review. *Science International*, (51): 51-73

Katz, SA., and Chatt, A. 1994. The use of hair as a biopsy tissue for trace elements in the human body. *NAHRES*, 22

Kennedy, GL., Pedro, MR., Seghieri, C., Nantel, G., Brouwer, I. 2007. Dietary diversity score is a useful indicator of micronutrient intake in non-breast-feeding Filipino children. *The Journal of Nutrition*, 137(2): 472-477

Kenya Demographic and Health Survey. 2009. Kenya National Bureau of Statistics, Nairobi, Kenya, & MEASURE DHS, ICF Macro, Calverton, Maryland, USA

Khan MJ., Ullah, U., Usuma, Lowe N., Broadley, M., Afridi, MZ., Zia, M., McArdle, HJ., Young S. 2017. Effect of agronomically biofortified zinc flour on zinc and selenium status in resource poor settings; a randomised control trial. *Proceedings of the Nutrition Society* 76, Issue OCE4 (Summer Meeting, 10–12 July 2017, Improving Nutrition in Metropolitan Areas)

Khoury, CK., Bjorkman, AD., Dempewolf, H., Ramirez-Villegas, J., Guarino, L., Jarvis, A., Rieseberg, LH., Struik, PC. 2013. Increasing homogeneity in global food supplies and the implications for food security. *Proceedings of the National Academy of Sciences*, 111: 4001-4006

Kikkert, J. and Berkelaar, E. 2013. Plant uptake and translocation of inorganic and organic forms of selenium. *Archives of Environmental Contamination and Toxicology,* 65: 458-465

Kishosha, PA., Galukande, M., Gakwaya, AM. 1999. Selenium Deficiency a Factor in Endemic Goiter Persistence in Sub-Saharan Africa. *American Journal of Public Health,* 89:1857-61

Kombo, K. 2003. Witchcraft: a living vice in Africa. *Africa Journal of Evangelical Theology*, 22.1

Kuona, P., Mashavave, G., Kandawasvika, GQ., Dzangare, J., Masanganise, M., Chandiwana, P., Munjoma, M., Nathoo, K., Stray-Pedersen, B. 2014. Serum Selenium Levels and Nutritional Status of School Children from an HIV Prevention Programme in Zimbabwe. *Journal of Tropical Diseases and Public Health*, 2:3

Lachat, C., Raneri, JE., Smith, KW., Kolsteren, P., Damme, P. V., Verzelenc, K., Penafiel, D., Vanhove, W., Kennedy, G., Hunter, D., Odhiambo, F. O., Ntandou-Bouzitou, G., Baets, B., Ratnasekera, D., Ky, HT., Remans, R., Termote, C. 2018. Dietary species richness as a measure of food biodiversity and nutritional quality of diets. *PNAS*, 115(1): 127-132

Lal, R. 2006. Enhancing crop yields in the developing countries through restoration of the soil organic carbon pool in agricultural lands. *Land Degradation & Development*, 17(2): 197-209

Lalentine, JL., Kang, HK., Spivey, GH. 1978. Selenium levels in human blood, urine, and hair in response to exposure via drinking water. *Environmental research,* 17: 347-355

Lavu, RVS., De Schepper, V., Steppe, K., Majeti, PNV., Tack, F., Du Laing, G. 2013. Use of selenium fertilizers for production of Se-enriched Kenaf (Hibiscus cannabinus): Effect on Se concentration and plant productivity. *Journal of Plant Nutrition and Soil Science*, 176(4): 634-639

Lawson, PG., Daum, D., Czauderna, R., Meuser, H., Härtling, JW. 2015. Soil versus foliar iodine fertilization as a biofortification strategy for field-grown vegetables. *Frontiers in Plant Science*, 6

Li, S., Banuelos, G.S., Wu, L., Shi, W. 2014. The changing selenium nutritional status of Chinese residents, *Nutrients*, 6: 1103-1114.

Lombeck, I., Kasparek., Bonnerman, B. 1978. Selenium content of human milk, cow's milk and cow's milk infant formulaes. *European Journal of Pediatrics*, 129: 139-145

Lu, J. and Holmgren, A. 2009. Selenoproteins. *The Journal of Biological Chemistry*, 284: 723–727

Luckett, BG., DeClerck, FA., Fanzo, J., Mundorf, AR., Rose, D. 2015. Application of the nutrition functional diversity indicator to assess food system contributions to dietary diversity and sustainable diets of Malawian households. *Public Health Nutrition*, 18: 2479-2487

Lyons, GH., Judson, GJ., Ortiz-Monasterio, I., Genc, Y., Stangoulis, JCR., Graham, RD. 2005. Selenium in Australia: selenium status and biofortification of wheat for better health. *Journal of Trace Elements in Medicine and Biology,* 19: 75–82

Lyons, GH.,Lewis, J., Lorimer, MF., Holloway, RE., Brace, DM., Stangoulis, JCR., Graham RD. 2004b. High-selenium wheat: agronomic biofortification strategies to improve human nutrition. *Food, Agriculture and Environment*, 2(1): 171-178

Lyons, GH., Stangoulis, JCR., Graham, RD. 2004a. Exploiting micronutrient interaction to optimize biofortification programs: The case for inclusion of selenium and iodine in the Harvest-Plus program. *Nutrition Reviews*, 62: 247-252

Maitima, JM., Olson, JM., Mugatha, SM., Mugisha, S., Mutie, IT. 2010. Land use changes, impacts and options for sustaining productitity and livelihood in the Basin of Lake Victoria. *Journal of Sustainable Development in Africa*, 12: 1520-5509

Makalle, AMP., Obando, J., Bamutaze, Y. 2008. Effects of land use practices on livelihoods in the transboundary sub-catchments of the Lake Victoria Basin. *African Journal of Environmental Science and Technology*, 2: 309-317

Mao, H., Wang, J., Wang, Z., Zan, Y., Lyons, G., Zou, C. 2014. Using agronomic biofortification to boost zinc, selenium, and iodine concentrations of food crops grown on the loess plateau in China. *Journal of Soil Science and Plant Nutrition*, 14: 459-470

Maobe, NS., Dyck EA., Mureithi JG. 1997. Screening of soil-improving herbaceous legumes inclusion into smallholder farming systems in Kenya – In: Soil fertility research for maize-based farming systems in Malawi and Zimbabwe. ISBN 970-648-006-4

Martin-Prevel, Y., Arimond, M., Allemand, P., Wiesmann, D., Ballard, T., et al. 2017. Development of a dichotomous indicator for population-level assessment of dietary diversity in women of reproductive age. Current Developments in Nutrition, *American Society for Nutrition*, 1 (12)

Masset, E., Haddad, L., Cornelius, A., Isaza-Castro, J. 2012. Effectiveness of agricultural interventions that aim to improve nutritional status of children: systematic review. *BMJ*, 344, d8222

Masters, WA., Bai, A., Herforth, A., Sarpong, DB., Mishili, F., Kinabo, J., Coates, JC. 2019. Measuring the affordability of nutritious diets in Africa: price indexes for diet diversity and the cost of nutrient adequacy. *American Journal of Agricultural Economics*, 100: 1285-1301

Mayland, BHF., Gough, LP., Stewart, KC. 1991. *Selenium mobility in soils and its absorption, translocation, and metabolism in plants*. Western U.S. Symposium on Selenium

Mehdi, Y., Hornick, J., Istasse, L., Dufrasne, I. 2013. Selenium in the Environment, Metabolism and Involvement in Body Functions. *Molecules*, 18: 3292-3311

Mengel, K. and Kirkby, EA. 1987. Principles of Plant Nutrition. International Potash Institute. ISBN 978-1-4020-0008-9

Micronutrient Initiative. 2009. Investing in the Future: A united call to action on vitamin and mineral deficiencies. 2009. Micronutrient Initiative, Flour Fortification Initiative, USAID, GAIN, WHO, The World Bank, and UNICEF, https://reliefweb.int/sites/reliefweb.int/files/resources/E7048CF2A553C171492576DB001FB2E2-Investing_in_the_future.pdf. Accessed on 2 May 2018

Miekeley, NM., Carneiro, TW., Silveira, CL. 1998. How reliable are human hair reference intervals for trace elements? *The Science of the Total Environment*, 218: 9-17

Mikkelson, RL., Page, AL., Bingham, FT. 1989. Factors affecting selenium accumulation by agricultural crops. *Soil Science Society of America*, 23: 65-94

Miller, N. and Yeager, R. 2018. Kenya: The quest for prosperity. Second Edition. Routledge. ISBN 13: 978-D-8133-8202-9

Ministry of Health, Republic of Kenya. 2013. National Iron and Folic Acid Supplementation Communication Strategy Nairobi, Kenya: Government of Kenya; @Government of Kenya

Mistry, HD., Broughton Pipkin F., Redman, CW., Poston L. 2012. Selenium in reproductive health. *American Journal of Obstetrics and Gynecology*, 206(1): 21-30

MoA. 2013. Economic Review of Agriculture [ERA]. https://books.google.co.uk/books?id=wplfnQAACAAJ. Accessed on 6 September 2018

MoH, 2018. Kenya national food fortification strategic plan: 2018 – 2022. http://www.nutritionhealth.or.ke/wp-content/uploads/Downloads/Food%20Fortification%20Strategic%20Plan%20Final%20Press%20Signed%20-%20Aug%202018.pdf. Accessed on 11 July 2019

MoH, KeMRI, KNBS. 2013. The Kenya National Micronutrient Survey 2011. http://www.nutritionhealth.or.ke/wp-content/uploads/Downloads/The Kenya National Micronutrient Survey 2011.pdf. Accessed on 3 May 2018

MoH. 2017. National Iron & Folic Acid Supplementation Communication Strategy 2013-2017. https://www.k4health.org/sites/default/files/ifas_national_comunication_strategy_2013-2017.pdf. Accessed on 2 January 2018

Moreno-Reyes, R., Egrise, D., Nève, J., Pasteels, JL., Schoutens, A. 2001. Selenium deficiency-induced growth retardation is associated with an impaired bone metabolism and osteopenia. *Journal of Bone and Mineral Research,* 16(8): 1556-1563

Murphya, SP., Gewab, C., Grillenberger, M. 2004. Adapting an international food composition table for use in rural Kenya. *Journal of Food Composition and Analysis,* 17: 523-530

National Academy of Sciences. 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. https://doi.org/10.17226/9810

Ne`ve, J. 1991. Methods in determination of selenium states. *Journal of trace elements and electrolytes in health and disease*, 5: 1-17Nogales, F., Ojeda, ML., Fenutrıa, M., Murillo, ML., Carreras, O. 2013. Role of selenium and glutathione peroxidase on development, growth, and oxidative balance in rat offspring. *Reproduction*, 146: 659-667

Neal, RH. 1995. Selenium. In Heavy Metals in Soils (B. J. Alloway, Ed.), Blackie Academic & Professional, London, pp. 260–283

Ning, Y., Wang, X., Wang, S., Zhang, F., Zhang, L., Lei, Y., Guo, X. 2015. Is It the Appropriate Time to Stop Applying Selenium Enriched Salt in Kashin-Beck Disease Areas in China? *Nutrients*. 7: 6195-212

Njoroge, AW., Baoua, I., Baributsa, D. 2019. Postharvest Management Practices of Grains in the Eastern Region of Kenya. *Journal of Agricultural Science*, 11(3)

OECD (Organisation for Economic Co-operation and Development). 2006. Trade, agriculture and development: Policies working together. Organisation for economic co-operation and development. ISBN-92-64-02200-7

Padmaja, K., Prasad, DDK., Prasad, ARK. 1989. Effect of selenium on chlorophyll biosynthesis in mung bean seedlings. *Phytochemistry*, 28:v3321-24

Pannier, F., Potin-Gautier, M. 2007. Determination of Organic and Inorganic Selenium Species Using HPLC-ICP-MS Application. Agilent Tech, 1-6. https://pdfs.semanticscholar.org/06f5/0253a32b2c521e4726e091884f501dd63da7.pdf. Accessed on 9 April 2018

Papp, LV., Lu, J., Holmgren A., Khanna, KK. 2007. From selenium to selenoproteins: synthesis, identity, and their role in human health. *Antioxidants & Redox Signaling*, 9: 775-806

Parkman, H. and Hultberg, H. 2002. Occurence and effects of selenium in the environment - a literature review. IVL Swedish Environmental Research Institute. http://agris.fao.org/agris-search/search.do?recordID=US201300081505. Accessed on 10 June 2018

Parr, RM., Crawley, H., Aldulla, M., Iyengar, GV., Kumpulainen, J. 1992. Human dietary intakes of trace elements: A global literature survey mainly for the period 1970-1991. Data listings and sources of information. NAHRES 12, Vienna, International Atomic Energy Agency

Peterson, PJ. and Butler, GW. 1962. The Uptake and Assimilation of selenite by higher plants. Australian *Journal of Biological Sciences*, 24(2): 175-178

Pingali, PL. 2001. CIMMYT 1999–2000 World maize facts and trends. Meeting world maize needs: Technological opportunities and priorities for the public sector. Mexico: CIMMYT

Poelman, MP., Steenhuis, IHM, De Vet, E., Seidell, JC. 2013. The Development and Evaluation of an Internet-Based Intervention to Increase Awareness About Food Portion Sizes: A Randomized, Controlled Trial. *Journal of Nutrition Education and Behavior*. 45: 701-707

Popkin, BM. 2002. An overview of the nutrition transition and its health implications: the Bellagio meeting. *Public Health Nutrition*, 5: 93-103

Preda, C., Vasiliu, I., Bredetean, O., Gabriela, C.D., Ungureanu, M.C., Leustean, E. L., Grigorovici, A., Oprisa, C., Vulpoi, C. 2016. Selenium in the environment: essential or toxic to human health? *Environmental Engineering and Management Journal,* 15: 913-921

Quagrainie, K., Dennis, J., Coulibaly, J., Ngugi, C., Amisah, S. 2009. Developing supply chain and group marketing systems for fish farmers in Ghana and Kenya. Technical Reports: Investigations 2007-2009

Ralston, NVC., Unrine, J., Wallschläger, D. 2010. Biogeochemistry and Analysis of Selenium and its Species. North American Metals Council, 1, 1-58. https://pdfs.semanticscholar.org/2478/928f08f55584e4e1928c2d7dc34f945ea256.pdf. Accessed on 28 March 2018

Rayman, MP. 2012. Selenium and Human Health. *The Lancet,* 379: 1256-1268

Rayman, MP. 2005. Selenium in cancer prevention: a review of the evidence and mechanism of action. *Proceedings of the Nutrition Society*, 64: 527-42

Rayman, MP. 2000. The importance of selenium to human health. *The Lancet*, 356 (9225): 233-241

Rayman, MP. 2002. The argument for increasing selenium intake. *Proceedings of the Nutrition Society*, 61: 203-215

Rayman, MP. 2008. Food-chain selenium and human health: emphasis on intake. *British Journal of Nutrition,* 100(2): 254-268

Reis, HPG., Barcelos, JP., Junior, EF., Santos, EF., Silva, VM., Moraes, MF., Putti, FF., Reis, AR. 2018. Agronomic biofortification of upland rice with selenium and nitrogen and its relation to grain quality. *Journal of Cereal Science,* 79: 508-515

Reuben, R., Slingerland, M., Nijhoff, H. 2006. The agro-food chains and networks for development. Springer Netherlands. ISBN: 978-1-4020-4592-9

Ros, G., Los, D., Bussink, W., Bindraban, PS. 2016. Selenium fertilization strategies for bio-fortification of food: an agro-ecosystem approach*. Plant and Soil*, 404(1)

Ruel, MT. 2003. Operationalizing dietary diversity: a review of measurement issues and research priorities. *The Journal of Nutrition*, 133: 3911-3926

Sahn, DE. 2015. The fight against hunger and malnutrition: the role of food, agriculture, and targeted policies. Oxford Scholarship Online. ISBN-13: 9780198733201

Sappington, KG. 2002. Development of aquatic life criteria for selenium: a regulatory perspective on critical issues and research needs. *Aquatic Toxicology*, 57: 101-113.

Sattar, N., Eatock, F., Fell, G.S., O'Reilly, D. 1997. Selenium: an acute phase reactant. Annals of Clinical Biochemistry, 19: 213-219

Save the Children. 2017. A Cost of the Diet analysis in Turkana County, Kenya: Central Pastoral Livelihood Zone. Nairobi, Kenya: https://pdfs.semanticscholar.org/de67/47b17c0402645b196fed931d5218cbd3db73.pdf Accessed on 4 May 2019

Schiavon, M., Acqua, S., Mietto, A., Pilon-Smits, EAH., Sambo, P., Masi, A., Malagoli, M. 2013. Selenium Fertilization Alters the Chemical Composition and Antioxidant Constituents of Tomato (Solanum lycopersicon L.). *Journal of Agricultural and Food Chemistry*, 61: 10542-10554

Senofonte, O., Violante, N., Caroli, S. 2000. Assessment of reference values for elements in human hair of urban schoolboys. *Journal of Trace Elements in Medicine and Biology*, 14(1): 6-13

Serna-Saldivar, SO. 2010. Cereal Grains: Properties, Processing, and Nutritional Attributes. Boca Raton: CRC Press

Severi, S., Bedogni, G., Manzieri, AM., Poli, M., Battistini, N. 1997. Effects of cooking and storage methods on the micronutrient content of foods. *European Journal of Cancer Prevention*, 6: 21-24

Sibhatu, KT., Krishna, VV., Qaim, M. 2015. Production diversity and dietary diversity in smallholder farm households. *Proceedings of the National Academy of Sciences*, 112(34): 10657-10662

Sive, AA., Subotzky, BF., Halan, H., Dempster, WS., Heese, HD. 1993. Red blood cell antioxidant enzyme concentration in kwashiorkor and marasmus. *Annals of Tropical Paediatrics,* 13: 33-38

Skorupa, J.P. 1998. Selenium poisoning of fish and wildlife in nature: lessons from twelve real- world examples. In: Frankenberger WT, Engberg RA (Eds) Environmental Chemistry of Selenium, Marcel Dekker, New York, 315-354

Soil Survey Staff. 2011. Soil Survey Laboratory Information Manual. Soil Survey Investigations Report No. 45, Version 2.0. R. Burt (ed.). U.S. Department of Agriculture, Natural Resources Conservation Service.

Sombroek, WG., Braun, HMH., Pouw, BJA. 1982. Exploratory Soil Map and Agro-Climatic Zone Map of Kenya, 1980. Scale: 1:1'000'000. Exploratory Soil Survey Report No. E1. Kenya Soil Survey Ministry of Agriculture - National Agricultural Laboratories, Nairobi, Kenya

Stamoulis, KG., Pingali, P., Shetty, P. 2004. Emerging Challenges for Food and Nutrition Policy in Developing Countries. *Journal of Agricultural and Development Economics*, 1: 154-167

Takeno, N. 2005. Atlas of Eh-pH diagrams: Intercomparison of thermodynamic databases. Geological Survey of Japan Open File Report No.419. http://www.eosremediation.com/download/Chemistry/Chemical%20Properties/Eh_pH_Diagrams.pdf. Accessed on 18 August 2019

Tan, J. 1989. The Atlas of Endemic Diseases and Their Environments in the People's Republic of China, Science Press, Beijing

Tanumihardjo, SA. 2008. Biofortification of staple crops: An emerging strategy to combat hidden hunger. *Comprehensive Reviews in Food Science and Food Safety,* 7: 329-334

Tapiero, H., Townsend, DM., Tew, KD. 2003. The antioxidant role of selenium and seleno-compounds. *Biomedicine and Pharmacotherapy*, 57(3): 134-144

Terry, N., Zayed, MA., De Souza, MP., Tarun, AS. 2000. Selenium in higher plants. *Annual Review on Plant Physiology and Plant Molecular Biology*, 51: 401-32

Tefera, T. 2012. Post-harvest losses in African maize in the face of increasing food shortage. *Food Security*. 4(2): 267–277

Terry, N., Zayed, AM., Souza, MP., Tarun, AS. 2000. Selenium in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biolog,* 51: 401-32

Tian, M., Hui, M., Thannhauser, TW., Pan, S., Li, L. 2017. Selenium-Induced Toxicity Is Counteracted by Sulfur in Broccoli (Brassica oleracea L. var. italica). *Plant Science*, Doi.org/10.3389/fpls.2017.01425

Tilman, D. and Clark, M. 2014. Global diets link environmental sustainability and human health. *Nature***,** 515: 518-522

Tinggi, U. 2008. Selenium: its role as antioxidant in human health. *Environmental Health and Preventive Medicine*, 13: 102-108

Tontisirin, K., Nantel, G., Bhattacharjeef, L. 2002. Food-based strategies to meet the challenges of micronutrient malnutrition in the developing world. *Proceedings of the Nutrition Society*, 61: 243-250

Townsend, R. 2015. Ending poverty and hunger by 2030: an agenda for the global food system (English). Washington, D.C.: World Bank Group. http://documents.worldbank.org/curated/en/700061468334490682/Ending-poverty-and-hunger-by-2030-an-agenda-for-the-global-food-system. Accessed on 5 May 2019.

Tulchinsky, TH. 2010. Micronutrient deficiency conditions: global health issues. *Public Health Reviews*, 32: 243-255

Turner, JC., Osborn, PJ., McVeagh, SM. 1990. Studies on selenate and selenite absorption by sheep ileum using an everted sac method and an isolate, vascularly perfused system. *Comparative Biochemistry and Physiology Part A: Physiology,* 95: 297-301

USAID (United States Agency for International Development). 2013. Feed the Future Zone of Influence Baseline Report: Kenya. The US Government's Global Hunger & Food Security Initiative. https://feedthefuture.gov/sites/default/files/resource/files/Kenya_Feed_the_Future_Baseline_Report.pdf. Accessed on 2 March 2018

Valença, AW., Bake, A., Brouwer, ID., Giller, KE. 2017. Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. *Global Food Security,* 12: 8-14

Van Dael, P., Davidsson, L., Ziegler, EE., Laurent BF., Barclay, D. 2002. Comparison of selenite and selenate apparent absorption and retention in infants using stable isotope methodology. *Pediatric Research*, 1: 71-75

Vanderpas, JB., Contempre, B., Duale, N.L., Goossens,, W., Bebe, N., Thorpe, R., Ntambue, K., Dumont, J., Thilly, CH., Diplock, AT. 1990. Iodine and selenium deficiency associated with cretinism in Northern Zaire. *The American Journal of Clinical Nutrition,* 52: 1087-93

Vendeland, SC., Deagen, JT., Butler, JA., Whanger, PD. 1994. Uptake of selen-ite, selenomethionine and selenate by brush border membranevesicles isolated from rat small intestine. *Biometals*,;7: 305-12

Vineyard, B. 2010. Plant Nutrition. *Forum American Bar Association*, 49, 27,1,183

Walingo, MK., Liwenga, ET., Kangalawe, RY., Madulu, NF., Kabumbuli, R. 2009. Perceived impact of land use changes and livelihood diversification strategies of communities in the Lake Victoria Basin of Kenya. *Journal of Agricultural Biotechnology and Sustainable Development*, 1: 69-78

Wang, J., Wang, Z., Mao, H., Zhao, H, Huang, D. 2013. Increasing Se concentration in maize grain with soil- or foliar-applied selenite on the Loess Plateau in China. *Field Crops Research*, 150: 83-90

Wang, Q., Yu, Y., Li, J., Wan, Y., Huang, Q., Guo, Y., Li, H. 2017. Effects of Different Forms of Selenium Fertilizers on Se Accumulation, Distribution, and Residual Effect in Winter Wheat-Summer Maize Rotation System. *Journal of Agricultural and Food Chemistry*, 65: 1116-1123

Welch, RM. 2001. Micronutrients, agriculture and nutrition; linkages for improved health and well being, In: Singh, K., Mori, S., Welch, RM., (Eds.), Perspectives on the Micronutrient Nutrition of Crops. Scientific Publishers (India), Jodhpur, India, pp. 247-289

Welch, RM. and Graham, RD. 2005. Agriculture: the real nexus for enhancing bioavailable micronutrients in food crops. *Journal of Trace Elements in Medicine and Biology*, 18: 299-307

White, PJ. and Broadley, MR. 2005. Biofortifying crops with essential mineral elements. *TRENDS in Plant Science*, 10: 12

White, PJ. and Broadley, MR. 2009. Biofortification of crops with seven mineral elements often lacking in human diets - Iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist*, 182(1): 49-84

WHO (World Health Organisation). 1996. Trace elements in human nutrition and health. Geneva. ISBN9241561734

WHO (World Health Organisation). 2004. Vitamin and Mineral Requirements in Human Nutrition. Report of a Joint FAO/WHO Expert Consultation; World Health Organization: Bangkok, Tailand; Geneva, Switzerland; ISBN9241546123

WHO (World Health Organisation). 2006. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. Multicentre Growth Reference Study Group. Geneva: World Health Organization, pp. 312

WHO (World Health Organisation). 2008. Indicators for assessing infant and young child feeding practices: conclusions of a consensus meeting held 6–8 November 2007 in Washington D.C., USA. Geneva (Switzerland): Retrieved from: https://apps.who.int/iris/bitstream/handle/10665/43895/9789241596664_eng.pdf;jsessionid=C5E33DC91C1F403B604500155849E4A9?sequence=1. Accessed on 23 April 2019.

WHO (World Health Organization) & International Atomic Energy Agency. 1989. Minor and trace elements in breast milk: report of a joint WHO/IAEA collaborative study. World Health Organization. http://www.who.int/iris/handle/10665/39678. Accessed on 24 December 2018

Willett W. (2013). Nutritional Epidemiology. Oxford University Press USA. ISBN 0199754039, 9780199754038

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., Garnett, T., Tilman, D., DeClerck, F., Wood, A., et al. 2019. Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. *The Lancet Commissions*, 393: 447-492

Williamson, C. 2005. The Different Uses of Food Composition Databases, Synthesis report No 2.

https://www.bezpecnostpotravin.cz/UserFiles/File/Kvasnickova2/EuroFIR_2.pdf. Accessed on 13 May 2018

Wolfram, AS., Arduser, F., Sharrer, E. 1985. In vivo intestinal absorption of selenate and selenite by rats. *Journal of Nutrition*, 115: 454-459

World Bank. 2009. Kenya Poverty and Inequality Assessment: Executive Summary and Synthesis Report. World Bank Group, 44190-KE, http://documents.worldbank.org/curated/en/425611468089394594/pdf/441900ESW0P0901IC0Dislosed071171091.pdf. Accessed on 5 May 2019

Yang, G., Wang, S., Zhou, R. and Sun, S. 1983. Endemic Selenium Intoxication of Humans in China. *American Journal of Clinical Nutrition*, 37: 872-881

Yang, G., Zhou, R., Yin, S., Gu, L.., Yan, B., Liu, Y., Li, X. 1989. Studies of safe maximal daily dietary selenium intake in a seleniferous area in China. I. Selenium intake and tissue selenium levels of the inhabitants. *Journal of trace elements and electrolytes in health and disease*, 3: 77-87

Yang, GQ. and Xia, YM. 1995. Studies on human dietary requirements and safe range of dietary intakes of selenium in China and their application in the prevention of related endemic diseases. *Biomedical and Environmental Sciences*, 8: 187-201

Zayed, A., Lytle, CM., Terry, N. 1998. Accumulation and volatilization of different chemical species of selenium by plants. *Planta,* 206: 284-92

Zhao, C., Ren, J., Xue, C., Lin, E. 2005. Study on the relationship between soil selenium and plant selenium uptake. *Plant and Soil*, 277(1–2): 197-206

Zhao, F. and McGrath, SP. 1994. Extractable sulphate and organic sulphur in soils and their availability to plants. *Plant and Soil*, 164(2): 243-250

Zotor, FB., Ellahi, B., Amuna, P. 2015. Applying the food multimix concept for sustainable and nutritious diets. *Proceedings of the Nutrition Society*, 74: 505-516

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SUPPLEMENTARY INFORMATION

Supplementary information 1: Moisture content of consumed foods

Food Item	Moisture content (%)	Food Item	Moisture content (%)
Fresh maize grains	54,5	Orange	86,5
Ugali	69,2	Mango	86,2
Rice raw	13,4	Blood fruit	88,1
Rice cooked	69,2	Avocado	75,8
Chapati	27,3	Sugarcane	77,4
Mandazi	26,6	Horned melon	95,7
Sorghum	40,2	Pineapple	87,4
Amaranthus seeds	26,6	Passion	81,9
wheat grains	13,4	Sandara	77,7
Pegion peas	31,8	Pawpaw	75,1
Fresh pegion peas	52,2	Lukewarts	86,6
Beans	31,8	Macadamia	45,4
Green grams cooked	33,1	Groundnuts	58,7
Fresh black beans	9,32	Egg boiled	90,6
Cow peas	44,0	Beef roasted	53,1
Potatoes	75,0	Beef fried	56,2
Arrowroot	72,6	Beef stewed	63,1
Cassava	64,7	Beef cooked	70,1
Yams	70,8	Beef raw	75,2
Sweet potatoes	69,7	Beef fried matumbo	71,2
Green bananas	71,5	Goat stewed	67,9
Eggplant	90,9	Goat fried	58,1
Pumpkin	83,9	Goat cooked	58,3
Pumpkin leaves	82,7	Goat raw	76,1
Amaranthus leaves	76,2	Pork raw	74,5
Cow pea leaves	82,2	Pork cooked	64,9
Soybean	9,30	Chicken	59,8
Kales	83,9	Green tea	75,4
Cabbage	88,7	Kanyuria	76,0
Arrowroot leaves	87,0	Stinging netles	64,7
Sweet potato leaves	82,0	White bread	31,4
Indigenous vegetables	76,7	Organ beef raw	81,2
Onion	88,2	Organ goat raw	81,1
Garlic	64,3	Sheep raw	75,1
Tomato	93,0	Sheep cooked	66,3
Guavas	73,4	Cooked cowpeas + potatoes	68,1
Lemon	88,2	Cooked potatoes + meat + peas	78,8

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PUBLICATIONS AND WORKING PAPERS

- Ngigi, PB., Du Laing, G, Masinde, PW., Lachat, C. 2019. Selenium deficiency risk in central Kenya highlands - an assessment from the soil to the body. Paper under review in *Journal of Environmental Geochemistry and Health*.
- Ngigi, PB., Lachat, C., Masinde, PW., Du Laing, G. 2019. Agronomic biofortification of maize and beans in Kenya through selenium fertilization. *Environmental Geochemistry and Health*. (Accepted for publication).
- Ngigi, PB., Du Laing, G, Masinde, PW., Lachat, C. 2019. Association of dietary diversity with the risk of dietary Se deficiency: a comparative analysis of central highlands and the lake basin in Kenya. Paper in preparation.
- Ngigi, PB., Lachat, C., Masinde, PW., Du Laing, G. 2019. Impact of selenium agronomic biofortification intervention on selenium intake adequacy among under-5 children and women in Kenya. Paper in preparation.
- Ngigi, PB, Verbeke, W., Pérez-Cuet, FJA. 2011. Assessment of actual food portion sizes in a sample of adolescents from Cochabamba (Bolivia). *Nutricion Hospitalaria*. 26 (5): 1195-1197

CONFERENCES

- ***Sept. 5-7, 2018*** **5th International Zinc Symposium**: **Improving Crop Production and Human Health**, University of Leuven - Belgium, ***Poster presentation***
- ***Aug. 30-31, 2018*** **Scientific Innovation for a Sustainable Development of African Agriculture**, University of Ghent - Belgium, ***Poster presentation***
- ***Aug. 13-17, 2017*** **5th International Conference on Selenium in the Environment and Human Health**, Karolinska Institutet, Stockholm - Sweden, ***Oral presentation***
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2016 – 2017 Julie Vandeput - Improving human selenium status in the Kenyan highlands through biofortification

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2015 – 2016 Monica Ercolani - Evaluation of Selenium levels in Kenyan soils, foods and bioaccumulation in hair